White Paper: Gut Sensory Modulation (GSM)

Chemosensory Receptors, Gut Hormones and Metabolic Homeostasis

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Introduction

Excess caloric reserves in the form of fat do not confer a commensurate sense of satiety. In fact, it appears that with increasing weight gain, individuals need to ingest a greater amount of calories to achieve a given level of satiation (11). In other words, with greater weight gain, satiety/appetite centers appear to become resistant to normal satiety signals and are reset at a higher threshold of caloric intake i.e., it takes more food to feel full.

While leptin, a hormone produced by the fat cell in proportion to fat stores, sets a chronic tone for satiety, day-to-day and meal-to-meal satiety (less hunger between meals) and satiation (less hunger as a result of meals) are controlled by acute signals, including neural and hormonal feedback signals from the gut (12). The field of satiety and satiation has undergone a recent explosion of knowledge mostly as a result of insights gleaned from experience with bariatric surgery. Indeed, surgical procedures that bypass the small intestine and deliver foodstuffs to the lower intestine are associated with increased satiety and elevations of gut hormones known to increase satiation and fullness, as well as signal meal cessation, resulting in dramatic weight loss and diabetes remission (13-21).

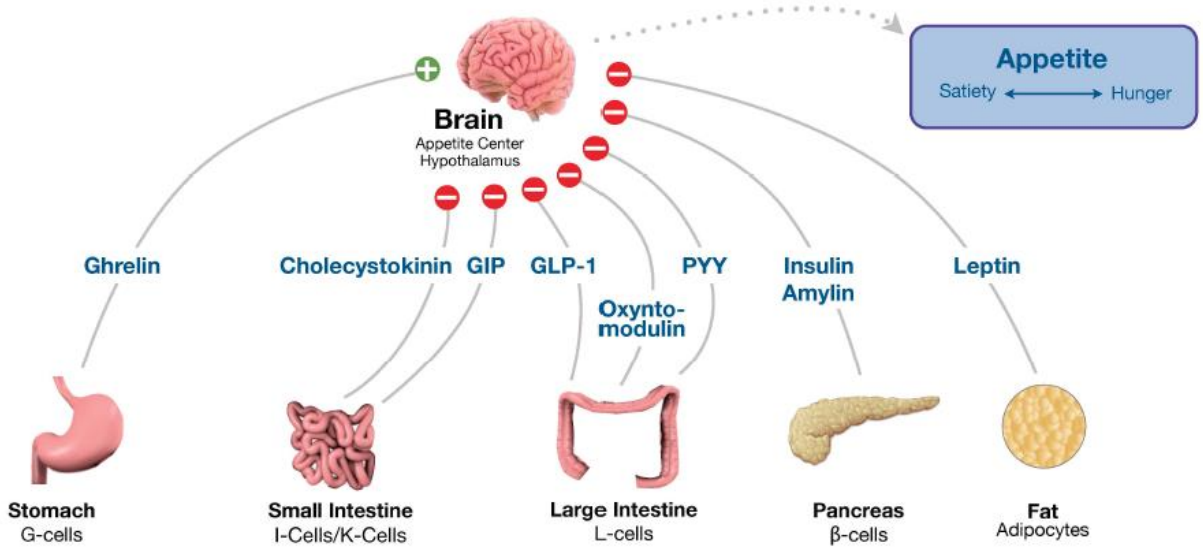
It has long been recognized that nutrients are powerful signals for the release of gut hormones, however the exact mechanism by which nutrients signal hormone release from enteroendocrine cells (EECs) is still not yet fully known. Recent published reports suggest that taste receptors for sweet, bitter, umami and others, similar to those present in taste buds on the tongue, are present on the luminal aspect of EECs. These receptors are expressed in the gut where they have direct contact with nutrients, providing a putative nutrient-sensitive mechanism for activation of EECs and gut hormone release (22-29).

This document reviews the available evidence, both supportive and otherwise, for the role of taste receptors in modulating gut hormone release from EECs. We will focus on human data where available. Finally, we will discuss the rationale for targeting gut taste receptors as a novel approach for weight management.

I. Enteroendocrine cells and gut hormones

EECs are specialized cells scattered throughout the gut epithelium. They represent a major endocrine organ(s) even though they account for approximately 1% of the epithelial cellular mass. Activity of EECs is influenced by numerous nutrient-related signals, including G protein-coupled receptor (GPCR) taste receptors, lipid sensitive GPCRs, changes in potassium channel conductance, and nutrient and amino acid transport (28). Eleven different EEC cell types have been recognized and more may yet be identified; some EEC cell types are quite sparse and thus less studied.

Figure 1. Food-related hormones control appetite and metabolism of food.



Among the most thoroughly studied EECs are the K- and L-cells, characterized by the hormones they secrete (**Figure 1)** (28). K-cells are chiefly located in the upper small intestine and are sparse, while L-cells are located throughout the gut with increasing density in the lower bowel. It is not clear whether L-cells behave identically in various parts of the gut (30). However, L-cells harvested from the mouse colon respond to the non-nutritive sweetener (NNS) sucralose, while those harvested from the proximal and mid-bowel do not (30).

K-cells secrete insulinotropic polypeptide (GIP). L-cells secrete the proglucagon products glucagon-like peptide 1 (GLP-1) and GLP-2, peptide YY (PYY), oxyntomodulin and glycentin. GLP-1 is an incretin (augments glucose-dependent insulin secretion). It slows gastric emptying, and like oxyntomodulin is also a satiety signal. GLP-2 is a gut growth factor, and glycentin has no well-defined physiologic actions. The long form of PYY secreted by L-cells (PYY_{1-36}) is hydrolyzed by DPP-IV to its active form PYY_{3-36}, a potent satiogenic hormone (31-33). L-cells in the upper small intestine also secrete cholecystokinin (CCK), a satiogenic peptide that causes gall-bladder contraction. Ghrelin, an orexigenic hormone that increases gastric motility is secreted from G-cells in in the stomach and small intestine (34).

II. Gut hormones act in concert to convey information about food intake

Secretion of gut hormones is differentially and rapidly influenced by ingestion of specific macronutrients (and perhaps micronutrients). For example, carbohydrate and protein are strong signals for GLP-1 secretion while carbohydrates and fat stimulate GIP and CCK release (19). It is common to discuss gut hormones as individual entities; however this neglects the fact that gut hormones are released in a coordinated fashion in response to food intake and act in concert rather than individually. Upon ingestion of a mixed meal, ghrelin and glucagon secretion is reduced, while secretion of CCK, GIP, GLP-1, PYY, oxyntomodulin, insulin and

amylin is increased. The resulting combined satiety signal is much more potent relative to that of any single hormone. This has been shown experimentally in rats, where combined administration of PYY, GLP-1 and the β-cell hormone amylin causes additive appetite suppression and weight loss (35). In humans, gastric bypass surgery is accompanied by elevations in multiple gut hormones including the L cell hormones (PYY, GLP-1, oxyntomodulin) (15, 18, 31, 36).

Because gut hormones are neuropeptides, they may act locally at afferent nerve endings or circulate in the bloodstream to activate receptors at distal locations such as the brain. The unique anatomy of the gut in which mesenteric veins drain into the hepatic portal vein likely facilitates distal signaling of gut hormones. Indeed, there are hepatic portal vein sensors that can detect GLP-1 (perhaps other hormones) and relay the GLP-1 signal via hepatic afferents to the hindbrain (37). It has been speculated that GLP-1's insulinotropic effect is largely mediated via this portal sensing mechanism, thus providing an explanation for the insulinotropic activity of DPP IV inhibitors, which cannot be accounted for merely by circulating GLP-1 concentrations (32, 38, 39).

Thus, it is helpful to think of gut hormones as feed-forward signals to the brain and body that communicate the nature and concentration of nutrients in the gut. Downstream signals then, through neural and hormonal pathways, direct the rapid and efficient storage of fuels (amino acids, sugars and fats) into macromolecules (protein, glycogen, and triglycerides). For example, glucose causes GLP-1 release, which augments insulin secretion and reduces glucagon secretion, favoring the disposal and storage of glucose as glycogen into skeletal muscle and liver. Likewise the uptake and deposition of fat into adipocytes is promoted by insulin and facilitated by GIP (40, 41). Finally, these integrated signals provide a "tally" of calories ingested and thus provide a commensurate signal for satiation and meal cessation.

III. Taste receptors

Taste receptors are chemosensory receptors that transmit and convey the perception of taste for bitter, sweet (sweet receptor), umami (savory, glutamic acid or glutamate salts), salt and sour tastants (42-45). Other putative taste receptors may exist for fats. Classically, we think of the taste receptors located on cells of taste buds on the tongue, however taste receptors also exist in other organs including the lung and EECs in the gut epithelium (22, 25, 44, 46-49).

Bitter (TAS2R), sweet (TAS1R2-R3) and umami (TASR1-R3) receptors are GPCR receptors, whereas ion channels detect salt and sour tastants (50). Sweet and umami receptors are heterodimers that share the TASR3 monomer. For GPCR taste receptors, signal transduction occurs via the specific G protein α-gustducin (51). Bitter receptors are the most prevalent taste receptors in taste buds on the tongue and represent a large heterogeneous family. Teleologically, it has been proposed that bitter receptors are most numerous as they protect against ingestion of a wide range of bitter toxins. A variety of non-nutritive sweeteners (NNS) have been developed that substitute for the nutritive agonist, sugar, at the sweet taste receptor but have minimal or no caloric value. These include stevia, aspartame, sucralose, neotame, acesulfame potassium, saccharin and others and are also referred to as artificial sweeteners (except for natural NNS such as stevia)

Recent reports suggest that taste receptors for sweet, bitter and umami tastants are expressed in EECs (22, 23, 30). The NNS sucralose causes dose-dependent release of GLP-1 in transformed human L-cells, which can be blocked by the sweet receptor antagonist, lactisole (22). Activation of bitter, sweet, umami, salt and sour receptors results in CCK and GLP-1 secretion, as does the temporal pattern of gut hormone release; bitter elicits a rapid burst of secretion while other tastants result in a sustained pattern of secretion (52). Interestingly, although both contain sucralose, Tagatesse® (Sucralose, Damhert, Heusden-Zolder, Belgium) and not Splenda® (Sucralose, Splenda, Washington, USA), caused GLP-1 release from L-cells. The reason for this is unknown, although the authors suggest that it may be due to the composition of the sweetener.

IV. Chemosensory receptor activation and gut hormone release in vivo

The first in vivo studies seemed to indicate that release of gut hormones is not dependent on gut chemo-sensation. An Intra-peritoneal glucose tolerance tests performed in rats following oral gavage of the NNS saccharin, acesulfame potassium, d-tryptophan, sucralose, or stevia showed that none of the NNS influenced blood glucose excursion (53). There was also no effect of any NNS on blood glucose levels in Zucker diabetic fatty rats. Finally, whereas oral glucose increased plasma GIP and GLP-1 levels, NNS failed to significantly increase plasma levels of these incretins. The authors concluded that carbohydrate-triggered incretin release from EECs occurs via a different mechanism than detection of "sweet taste" in the tongue.

In keeping with these results, Ma et al (54) studied the effect of intragastric infusion of sucrose, sucralose, or saline on blood glucose, plasma insulin, GLP-1, and GIP, and gastric emptying. Only sucrose resulted in an increase in blood glucose, GLP-1, and GIP, and a delay in gastric emptying. The authors concluded that intragastric infusion of the NNS, sucralose does not stimulate insulin, GLP-1, or GIP release or slow gastric emptying in healthy humans.

In a study that began to change our understanding of the function of taste receptors in the gut, Brown et al (55) showed that stimulation of gut taste receptors in the presence of a nutrient (glucose) results in a synergistic effect on gut hormone release. Healthy volunteers underwent two oral glucose tolerance tests after drinking diet soda or carbonated water. Compared to carbonated water, diet soda augmented GLP-1 release in the absence of significant differences in insulin or glucose. The authors concluded that the NNS contained in the soda (acesulfame potassium and sucralose) synergize with glucose to enhance GLP-1 release in humans; and that this effect may be mediated by stimulation of sweet taste receptors on L-cells by NNS.

Gerspach and colleagues (46, 56) employed a different approach in which lactisole, a sweet receptor blocker, was used to assess the role of the T1R2/T1R3 (sweet receptor) in GLP-1, PYY and CCK release in response to intragastric administration or intraduodenal perfusion of nutrients **(Figure 2)**. Subjects received either an intragastric or intraduodenal glucose solution or a mixed liquid meal with or without lactisole. During glucose perfusion, lactisole induced a significant reduction in GLP-1 and PYY, but not CCK. However, there was no effect of lactisole on any parameter during a liquid meal; the reason for this is unknown. In addition, intragastric lactisole was more effective at suppressing the hormonal response to glucose than

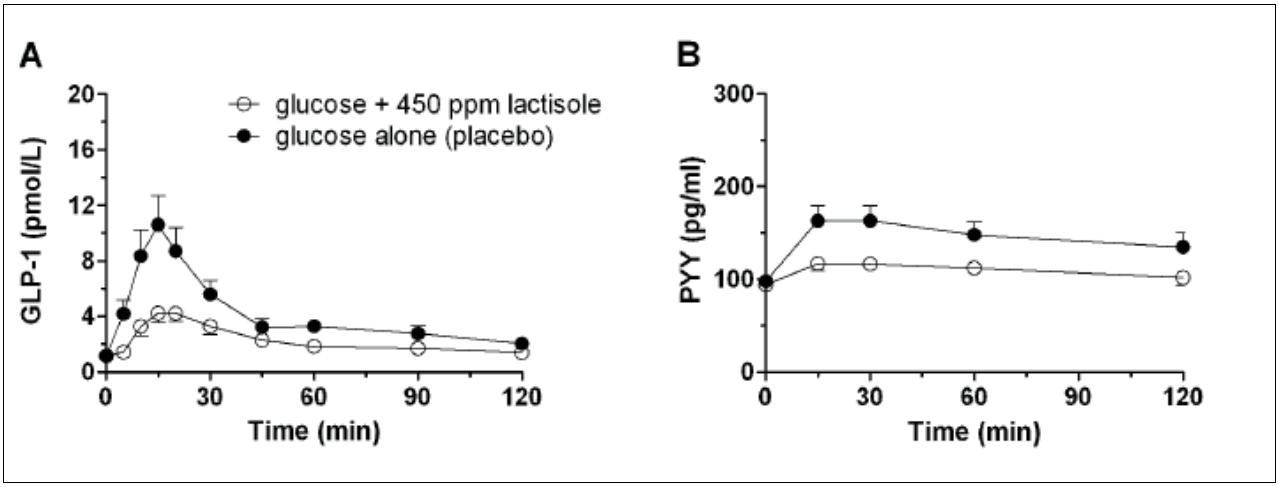


Figure 2. From Gerspach et al (2011). Sweet receptors partially mediate the GLP-1 and PYY response to glucose. Figure 1 Plasma concentrations of GLP-1 and PYY after an intragastric load of 75 g glucose, with or without 450 ppm lactisole. Whereas GLP-1 and PYY secretions were significantly (P = 0.007 and 0.012) reduced by lactisole in response to the intragastric glucose load, no effect was observed in response to the mixed liquid meal. Plasma CCK levels were not affected by lactisole, neither after intragastric glucose nor after mixed liquid meal administration. The intragastric infusions were administered at t = 0 min. Data are expressed as mean ± SEM. Reported data are from 26 healthy subjects (13 male and 13 female) with complete datasets.

intraduodenal lactisole. The authors concluded that the T1R2/T1R3 sweet receptor is involved in glucose-dependent secretion of satiation peptides but that the receptor is not alone responsible for peptide secretion.

It has been proposed that sweet receptor activation augments expression of the sodium-dependent glucose transporter (SGLT-1) in the intestinal lumen, to increase carbohydrate absorption (57). This does not appear to be the case, as Ma et al (58) showed no effect of sucralose on glucose transport across the duodenum.

These studies suggest that taste receptor activation alone is not sufficient to augment gut hormone secretion. However, **taste receptor activation in the presence of the cognate nutrient (such as sucrose or dextrose) appears to be synergistic for gut hormone release.** This observation is consistent with other nutrient driven hormone release systems. For example, the pancreatic β-cell both senses and metabolizes glucose. Insulin secretion from β-cells requires alteration of the ADP/ATP ratio via glucose metabolism through the glycolytic pathway. However activation of glucokinase, the β-cell glucose sensor, can synergistically augment glucose driven insulin secretion (59). Speculatively, gut hormone secretion may follow a similar biologic principle: nutrient chemo-sensation may not be sufficient to cause hormone secretion, but chemo-sensation may augment nutrient-driven hormone secretion.

V. Effects of NNS on in vivo gut hormone secretion, glucose metabolism and satiety

There has been a great deal of discussion about the role of NNS as a potential cause of excessive weight (60-63). In this regard, several theories underlying the putative role of NNS include 1) NNS may dissociate sweetness from calories, thus interfering with the physiological responses that control energy homeostasis; 2) NNS may

alter the intestinal environment, affecting gut microbiota and triggering inflammatory processes associated with metabolic disorders; or 3) may affect glucose absorptive capacity and glucose homeostasis by interacting with novel sweet-taste receptors in the gut. Although controversial, the literature suggests that NNS are weight neutral.

To test whether NNS influence glucose metabolism, Gregersen et al (64) conducted a study in which patients with type 2 diabetes were given a standard test meal supplemented with the NNS, stevioside, or maize starch (control). Stevioside reduced the area under the curve (AUC) for the glucose response by 18% and increased the insulinogenic index (the ratio of the AUCs for insulin and glucose responses) by approximately 40%. Stevioside also tended to decrease glucagon levels, but did not significantly alter the AUC for insulin, GLP-1, or GIP. The authors concluded that the NNS stevioside reduces postprandial blood glucose levels in patients with type 2 diabetes, indicating beneficial effects on glucose metabolism.

Two studies have examined the effects of chronic administration of NNS on glucose control in adults with type 2 diabetes. One study reported the effects of 16 weeks of rebaudioside A (a steviol glycoside used in sugar substitutes) compared to placebo (65) and another investigated the effect of daily administration of high doses of sucralose (approximately three times the estimated acceptable daily intake) for three months on glycemic control (66). There were no changes in any of the measures reported in either study, including blood pressure, body weight, fasting lipids, HbA1C, fasting glucose, insulin, and C-peptide, and no differences in hypoglycemic episodes. Together, these studies suggest that sustained NNS consumption for up to four months did not alter glucose metabolism or other metabolic parameters in patients with type 2 diabetes.

A recent study by Rogers et al (67) studied the effects of the NNS, aspartame, on motivation to eat and food intake in healthy adult volunteers. The results of this study provide a potential explanation for contradictory theories of NNS on metabolic parameters. There was clear evidence for a prominent post-ingestive inhibitory action of aspartame on appetite: consumption of aspartame capsules (at doses equivalent to several cans of soft drink) reduced subsequent food intake and, to a lesser extent, motivation to eat, while consumption of aspartame sweetened water did not. Although the mechanism for the reduction in appetite is unknown, the authors propose that the release of CCK by phenylalanine, a constituent of aspartame, may be involved. The authors propose that the response to consuming aspartame is determined by at least two interacting influences, an inhibitory post-ingestive effect and a stimulatory effect of its sweet taste on appetite. The relative potency of these influences may be modified by other features of the aspartame-sweetened food or drink (e.g., its nutrient content).

Anton et al (68) tested the effect of preloads containing stevia, aspartame, or sucrose on food intake, satiety, and postprandial glucose and insulin levels. On three different days, healthy lean and obese adults received preloads containing stevia (290 kcal), aspartame (290 kcal), or sucrose (493kcal) before the lunch and dinner meal. Stevia preloads significantly reduced postprandial glucose levels compared to sucrose and reduced postprandial insulin levels compared to both aspartame and sucrose. When consuming stevia and aspartame preloads, participants did not compensate by eating more

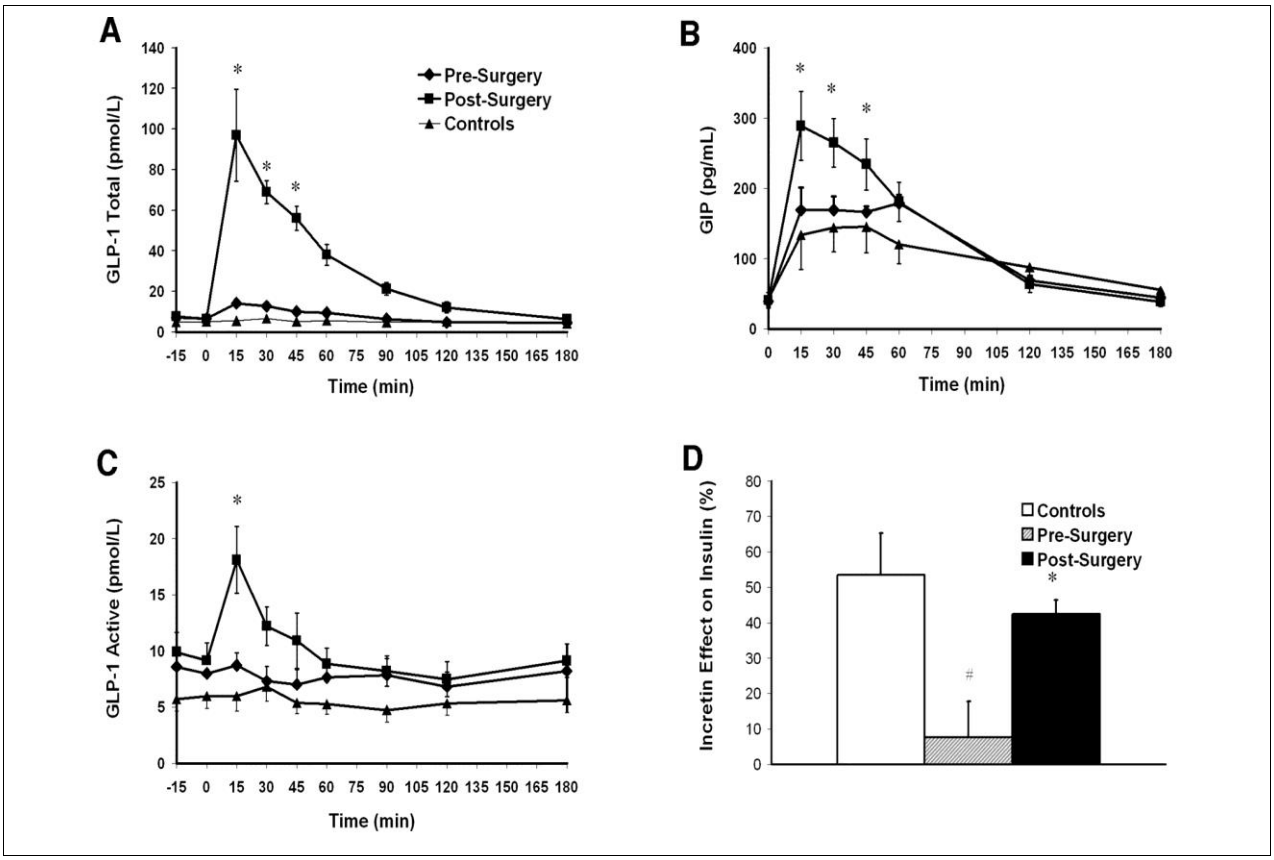
at either their lunch or dinner meal and reported similar levels of satiety compared to when they consumed the higher calorie sucrose preload. The authors concluded that some NNS may enhance satiation signals.

These studies indicate that the effects of NNS on appetite and body weight is complicated by ingestive feedback signals (taste) and the presence of nutrients. Although NNS can reduce food intake and appetite by influencing release of gut hormones, taste can act as a feed-forward signal, driving increased intake. Finally, NNS do not appear to have adverse effects on glucose control or related parameters in adults with type 2 diabetes.

VI. Rationale for targeting gut chemosensory receptors to modulate body weight

The dramatic results of bariatric surgery for weight loss and diabetes remission have brought attention to the role of the gut in regulating energy and glucose homeostasis. The Roux-en-Y gastric bypass procedure is the most common form of bariatric surgery performed with over 200,000 procedures a year in the US alone. With this procedure, the stomach and proximal small

Figure 3. From Laferrère et al (2007). Exaggerated incretin response to oral glucose post-bariatric surgery (Roux-en-Y) implicates enhanced luminal L-cell stimulation as a key contributor to rapid diabetes resolution. Total GLP-1 (*A*), GIP (*B*), and active GLP-1 (*C*) levels during OGTTs in patients before and after RY-GBP (*A*) and in control subjects and incretin effect on insulin secretion (*D*) in control subjects, patients before RY-GBP, and patients after RY-GBP. The incretin effect was calculated by comparing the insulin response to oral and matched IV glucose loads. Data are means ± SEM. **P* < 0.05 compared with patients before RY-GBP. #*P* < 0.05 compared with control subjects.



intestine are bypassed in such a way that ingested food makes first contact with the bowel at the level of the ileum, bypassing the duodenum and jejunum. This results in an exaggerated rise in meal driven gut hormone release **(Figure 3)** (15, 18, 69-72). Interestingly, another non-surgical procedure accomplishes a similar net qualitative result by endoscopically inserting a duodenal-jejunal bypass sleeve (a 60 cm impermeable fluoropolymer sleeve such as the EndoBarrier™ Gastrointestinal Liner, GI Dynamics, Inc., Lexington, MA), at the level of the duodenum, which shunts food from the stomach to the lower jejunum, artificially bypassing the duodenum. As reported by GI Dynamics, placement of the Endobarrier™ results in rapidly enhanced meal-driven gut hormone secretion, weight loss and amelioration of glucose metabolism in type 2 diabetic patients (73, 74).

Given that the gastrointestinal sleeve procedure, unlike surgery, is not associated with surgical damage to organs or nerves, these results speak to a larger effect of food on gut hormone release that could be due to the direct presentation of food to the lower bowel, and/or the effect of bypassing the duodenum and jejunum, the so-called “proximal bowel exclusion” theory (36), although recent publications have questioned the validity of the “proximal bowel exclusion” thesis (75). Other approaches that deliver nutrients to the lower bowel are also associated with increased GLP-1 release. For example, use of α-glucosidase inhibitors in humans is associated with a shift in the absorption of monosaccharides from the duodenum to the lower jejunum and ileum and is associated with increases in GLP-1 and GIP (76).

In summary, the data strongly suggest that delivery of nutrients to the lower bowel is associated with augmented gut hormone release. Moreover, nutrient-driven hormone secretion can be augmented by non-nutritive agonists of nutrient chemosensory or taste receptors (46, 55). Given that non-nutritive agonists can be more potent than nutrient agonists at the chemosensory receptor level (e.g. sucralose is 600 times sweeter than sucrose), the use of non-nutritive agonists to augment meal-driven gut hormone secretion may be a useful approach for weight management. Because gut chemosensory receptors are cell surface receptors located in the epithelial lining of the gut, taste receptor agonist can exert desired biological actions without being absorbed into the bloodstream. This reduces the potential for off-target adverse events.

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