Incretins in obesity and diabetes

Chee W. Chia and Josephine M. Egan

Intramural Research Program, National Institute on Aging, National Institutes of Health, Baltimore, Maryland, U.S.A.

Corresponding author contact information: Chee W. Chia, M.D., National Institute on Aging, Intramural Research Program, 3001 S. Hanover Street, NM-533, Baltimore, MD 21225. e-mail: chiac@mail.nih.gov

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Abstract

Incretins are hormones secreted from enteroendocrine cells after nutrient intake that stimulate insulin secretion from β cells in a glucose-dependent manner. Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are the only two known incretins. Dysregulation of incretin secretion and actions are noted in diseases such as obesity and diabetes. In this review, we first summarize our traditional understanding of the physiology of GIP and GLP-1, and our current knowledge of the relationships between GIP and GLP-1 and obesity and diabetes. Next, we present the results from major randomized controlled trials on the use of GLP-1 receptor agonists for managing type 2 diabetes, and emerging data on treating obesity and prediabetes. We conclude with a glimpse of the future with possible complex interactions between nutrients, gut microbiota, the endocannabinoid system, and enteroendocrine cells.

Introduction

The study of incretins has undergone tremendous growth over the past 50 years culminating in the use of incretin-based therapy for managing and treating two of the most pressing global public health crises: obesity and type 2 diabetes. The ability of crude extract from porcine upper intestine to lower blood glucose in humans was first reported in 1902.[1, 2] In 1932, La Barre and colleagues coined the *incretin* concept to describe as yet unknown humoral factors released from the intestine in response to a meal that lowered blood glucose.[3, 4] However, a quantitative demonstration of this phenomenon was not possible until the development of radioimmunoassay technology to assay circulating insulin three decades later.[5] McIntyre and colleagues observed that for the same amount of glucose, the oral route of administration induced a much greater insulin secretion than did the

intravenous route.[6, 7] The first incretin was described in 1973 when glucose-dependent insulinotropic polypeptide (GIP) was found to potentiate glucose-induced insulin secretion.[8] Glucagon-like peptide-1 (GLP-1), the second incretin, was discovered later in 1987.[9-11] In 1979, Creutzfeldt gave the criteria necessary to classify a substance as being an incretin: (1) has to be a gastrointestinal factor, (2) must be released by nutrients, and (3) must stimulate insulin secretion in a glucose-dependent manner at physiological levels.[12] To date, GIP and GLP-1 are the only two enteroendocrine hormones that satisfy these criteria.

In this report, we first review the classical view of the physiology of GIP and GLP-1 and the enteroendocrine cells that secrete them. We then summarize the current knowledge of the relationships between GIP and GLP-1 with obesity and diabetes, and we follow with a review of the results from major randomized controlled trials on the use of GLP-1 receptor (GLP-1R) agonists for managing type 2 diabetes and obesity. The recent advances in the area of dual incretin (GLP-1 and GIP) receptor co-agonism is also addressed. We conclude with a glimpse of where this exciting field is headed with the latest findings in the possibility of the same enteroendocrine cell switching hormone expression pending local cues; and the likely complex interactions among nutrients, gut microbiota, endocannabinoid system and enteroendocrine cells.

Physiology of incretins

Where do GIP and GLP-1 come from and what do they do? The classical view is that when digested food reaches the intestine, it stimulates GIP and GLP-1 secretion from K and L cells respectively. GIP and GLP-1 in turn stimulate glucose-dependent insulin secretion from β cells. Incretins are estimated to account for 50-65% of total insulin secretion after a meal.[13, 14]

Glucose-dependent insulinotropic polypeptide

Where is GIP found and how is it secreted? GIP is a 42-amino acid peptide first isolated from intestinal extract of pigs in 1971 and was initially named gastric inhibitory peptide for its property in dogs of inhibiting acid and pepsin secretion.[15] Later, GIP was found to potentiate glucose-induced insulin secretion in rodents and humans, which was considered to be the more important function of the hormone; hence the alternative name of "glucose-dependent insulinotropic polypeptide" was introduced.[8, 16] GIP is localized to enteroendocrine K cells mainly within the crypts and mid-zones of glands in the duodenum and to a lesser extend in the jejunum.[17-19] The K-cell density in human duodenal mucosa has been estimated at about 13 per 1000 total duodenal cells.[20] In addition to enteroendocrine cells, GIP protein has also been found in mammalian pancreatic α cells in the form

of $GIP_{1\text{-}30NH2}$ and is speculated to have a paracrine effect modulating islet development and function.[21] GIP gene expression has been detected in mammalian salivary glands, eye, and brain.[22-25] GIP secreted from cells outside of the intestinal tract is unlikely to contribute significantly to the circulating GIP levels.

In the enteroendocrine K cells, GIP secretion is regulated by intraluminal nutrients, neural stimuli and hormones. Glucose, fat, and protein given orally or intraduodenally increase GIP secretion in a dose-dependent manner.[26-32] Fat was found to be a more potent GIP secretagogue than isocaloric glucose.[33] Exactly how GIP secretion is regulated is still an area of active research. Perfusion studies of isolated rodent intestine showed that K cells detect carbohydrates *via* the sodium-dependent glucose transporter 1 (SGLT-1).[34, 35] GIP secretion is inhibited by the SGLT inhibitor phloridzin,[35] while increased by alpha-methylglucopyranoside (αMG), a substrate of SGLT-1.[36] Taste receptor subunit α-gustducin, a key G-protein involved in taste sensing, was discovered in human duodenal L cells and L/K cells.[37] Up to 75% of enteroendocrine cells in duodenum contain both GIP and GLP-1 (K/L cells).[38] More than 90% of L cells contain α-gustducin, but <50% of K cells did so.[37] Furthermore, α-gustducin knockout mice are characterized by deficiencies in GIP and GLP-1 secretion with associated decreased in insulin responses and impaired glucose tolerance.[37, 39] Glucose and low-calorie sweeteners were reported to induce GIP secretion from enteroendocrine L cells, the NCI-H716 cell line and GLUTag cells.[37, 39] However, human studies investigating possible induction of GLP-1 and GIP secretion by low-calorie sweeteners have shown negative results. Most human studies have been single exposure experiments in which low-calorie sweetener is given once in the form of diet soda.[40-43] Recently, in an observational study, regular consumption of low-calorie sweeteners was associated with greater increase in GIP secretion following an oral glucose load in humans.[44] Direct links between low-calorie sweetener use and enteroendocrine hormones in humans has not been systematically investigated, and interactions between low-calorie sweetener and gut microbiota may be involved.[45]

Dietary fat, through lipid receptors such as $G_{\alpha q}$-coupled lipid receptors (GPR40 and GPR120) and $G_{\alpha s}$-coupled lipid receptors (GPR119), have also been shown to play an important role in regulating incretin secretion.[46-48] Additionally, the autonomic nervous system plays a role in regulating GIP secretion because vagotomy and pyloroplasty are associated with higher GIP secretion.[49] However, altered gastric emptying following vagotomy or pyloroplasty as a cause of altered GIP secretion cannot be ruled out.[50] GIP secretion may also be under hormonal influence. Somatostatin-containing D-cells are located in close proximity to K and L cells and somatostatin has been shown to inhibit GIP secretion *in vitro*,[51] and *in vivo*.[52, 53] Insulin and glucagon infusion has been

shown to reduce intraduodenal and oral glucose-stimulated GIP secretion.[54, 55] Recently, cannabinoid receptors (CBRs) were shown to exert tonic control over GIP secretion in humans because a CBR agonist significantly increased the fasting levels of GIP in healthy men thus raises the possibility that gut hormones are influenced by endocannabinoids.[56]

Once secreted into the circulation, GIP is degraded by dipeptidyl peptidase-4 (DPP4), which cleaves the first two amino acids (Tyr and Ala) at the N-terminus of GIP into a biologically inactive metabolite.[57, 58] DPP4 is also bound to endothelial cells of blood vessels of gut and liver, and to lymphocytes and is present in the circulation in soluble form.[59, 60] The half-life of intact GIP is 5–7 min in humans.[57] Kidney is the major site of GIP elimination.[56, 61] Intact biologically active GIP levels in both healthy subjects and diabetic subjects are similar at about 55% of the "total" GIP concentrations after a mixed meal ingestion.[62]

Biological actions of GIP. Once secreted, GIP activates specific GIP receptors (GIPR) on target tissues to induce physiological effects. GIPR gene expression has been found in pancreas, stomach, small intestine, adipose tissue, adrenal cortex, pituitary, heart, testis, endothelial cells, bone, trachea, spleen, thymus, lung, kidney, thyroid, and different regions of the central nervous system.[50, 59, 60] In humans, high concentration of GIPR expression is found in β, α, and pancreatic polypeptide (PP) cells.[63, 64] Global GIPR gene knockout (*Gipr*$^{-/-}$) mice exhibit impaired oral glucose tolerance.[65] However, pancreatic β cell-specific *Gipr*$^{-/-}$ mice on low-fat diet, were reported to have lower meal-related insulin secretion, decreased adipocyte mass, and better insulin sensitivity and glucose tolerance when compared to controls. On high-fat diet, these β cell-specific *Gipr*$^{-/-}$ mice exhibited similar insulin profiles, glucose tolerance and adipocyte mass compared to those of control mice.[66]

The glucose-dependent property of GIP regulation of insulin secretion is well documented using hyperglycemic clamps. Under basal euglycemic state with plasma glucose around 5 mM (90 mg/dL), GIP infusion did not induce insulin secretion. With progressive hyperglycemia, GIP stimulated insulin secretion occurred in a glucose concentration-dependent manner. Insulin secretion progressively increased when plasma glucose was increased from basal euglycemic level to 54 mg/dl above basal followed by 143 mg/dl above basal.[67] Furthermore, GIP released in response to the oral ingestion of fat does not stimulate insulin secretion unless simultaneous intravenous glucose is given to increase plasma glucose levels.[68, 69] In addition to inducing insulin secretion from β cells, GIP has been shown to increase glucagon secretion from pancreatic α cells. In isolated perfused rat pancreas, GIP increased glucagon secretion at glucose concentration less than 5.5 mM (100 mg/dL), while it increased insulin secretion at glucose levels greater than 5.5 mM.[70] Similar

results have been reported In healthy humans where GIP was found to dose-dependently stimulate glucagon secretion at basal euglycemia.[71] In type 2 diabetes however, GIP administered at concentration 5 times that of physiological levels, increased glucagon secretion which offset any glucose-lowering effects of GIP through insulin secretion.[63]

GIP has other physiological effects in addition to its insulinotropic action as suggested by the presence of GIPR on rat adipocytes and 3T3-L1 cells.[72] GIP has been shown to increase plasma triglyceride clearance following meals, to increase lipoprotein lipase activity, and to promote fat storage by adipocytes.[73-75] Blocking GIP signaling by genetic means as occurs in *Gipr*$^{-/-}$ mice causes preferential oxidation of fat and results in less triglyceride deposition in liver, which eventually contributes to suppression of hepatic glucose output and improvement in insulin sensitivity.[75, 76] However, recent data suggest that GIP may have anti-obesity effects. Transgenic mice overexpressing GIP were shown to not only have improved β cell function and glucose tolerance, but also have enhanced insulin sensitivity, and were protected from high-fat diet-induced obesity.[77] Furthermore, in human adipose tissues, GIPR gene expression was negatively correlated with adiposity and positively correlated with insulin sensitivity.[78] The molecular mechanisms of action of GIPR have been reviewed in detail.[79, 80]

Glucagon-like peptide-1

Where is GLP-1 and how is it secreted? GLP-1 is a 30 amino-acid peptide first discovered in anglerfish in 1982.[9, 10, 81] In 1987, meal-induced GLP-1 secretion was found in humans, rats and pigs.[9-11, 81] GLP-1 exists in two equally bioactive forms, glycine-extended GLP-1 (GLP-1 [7–37]) and amidated GLP-1 (GLP-1 [7–36]).[82, 83] GLP-1 is localized to L cells mainly within the crypts and mid-zones of glands with increasing density from the duodenum to the colon.[84] In addition to enteroendocrine L cells, GLP-1 protein has also been found in the nucleus of the solitary tract of the brain stem of rats, monkeys and humans, and in taste cells within taste buds.[85-87] Additionally, GLP-1 production occurs in α cells within islet of Langerhans.[88]

Similar to GIP, GLP-1 secretion from enteroendocrine L cells is regulated by intraluminal nutrients, neural stimuli and hormones, and, similar to GIP, the underlying mechanisms of its secretion are still an area of active research. Oral carbohydrates and fat induce GLP-1 secretion whereas protein does not appear to be as effective.[89] Following the ingestion of nutrients, a rise in the plasma concentration of GLP-1 is observed within minutes.[90] In healthy subjects, fasting levels of plasma GLP-1 range from 5–10 pmol/L and increase by two- to three-fold after meal ingestion.[91]

GLP-1 levels peak about 20 min after oral glucose and about 60 to 90 min after mixed meal ingestion, and the levels subsequently gradually decline toward fasting levels.[60, 92]

Similar to GIP, glucose-induced GLP-1 secretion involves SGLT-1, and GLP-1 secretion is ablated by SGLT inhibitor phlorizin.[93] Taste receptor subunit α-gustducin, a key G-protein involved in taste sensing, was discovered in human duodenal L cells and L/K cells. As mentioned earlier, more than 90% of L cells contained α-gustducin, and α-gustducin gene knockout mice are characterized by deficiencies in GIP and GLP-1 secretion with associated decrease in insulin responses and impaired glucose tolerance.[37, 39] Glucose and low-calorie sweeteners was reported to induce GLP-1 secretion from enteroendocrine L cells, and from the NCI-H716 cell line and GLUTag cells.[37, 39] However, similar to GIP, human studies investigating possible induction of GLP-1 secretion by low-calorie sweeteners have shown negative results. Several G protein-coupled receptors (GPCRs) have been identified in L cells: GPR40, GPR41, GPR43, GPR 119, and GPR120 are a few of the notable ones.[89] However, the role of these GPCRs in regulating GLP-1 secretion and their therapeutic potential for regulating GLP-1 secretion in humans are not clear and is an area of active research.[89] Autonomic nervous system also plays a role because both vagotomy and pharmacological ablation of vagus nerve by muscarinic receptor antagonists abolished nutrient-induced GLP-1 secretion.[94, 95] Hormones also regulate GLP-1 secretion. For example, somatostatin inhibits GLP-1 secretion.[96] Intravenous infusion of GIP does not stimulate GLP-1 release in humans and at supra-physiological doses it actually leads to suppression of GLP-1 secretion.[63, 97, 98]

Just as with GIP, once secreted, GLP-1 is rapidly degraded by DPP4.[58] The half-life of GLP-1 is only 1–2 min.[58, 99] DPP 4 cleaves the N-terminal dipeptides (His^7–Ala^8) from GLP-1 (7–36) and renders the resulting major metabolite GLP-1 (9–36) insulinotropically inactive.[58-60, 100] Only 10-15% of endogenously released GLP-1 reaches the systemic circulation.[82] Intact biologically active GLP-1 levels in both healthy subjects and diabetic subjects are similar at about 40-50% of the "total" GLP-1 concentrations after a mixed meal ingestion or oral glucose challenged.[56, 62] And again, analogous to GIP, GLP-1 and its metabolites are rapidly cleared *via* the kidneys.[101]

Biological actions of GLP-1. Once secreted, GLP-1 exerts its activities through the GLP-1 receptor (GLP-1R) in multiple tissues including the pancreas, kidney, heart, lung, adipose and smooth muscle, as well as in specific nuclei in the central nervous system. The widespread distribution of the GLP-1R throughout different tissues suggests that GLP-1 has other physiological effects in addition to glucose regulation. In the pancreas, GLP-1R is expressed on β, δ, and likely a subset of α cells. [102-104] GLP-1 stimulates insulin secretion in a glucose concentration dependent manner at glucose level

above 4.3 mmol/L (77 mg/dL).[105] Furthermore, GLP-1 also increases insulin gene transcription, insulin biosynthesis, and increases β-cell mass by stimulating β-cell proliferation and suppressing β-cell apoptosis, at least in young rodents.[106, 107] Unlike GIP, GLP-1 is a strong inhibitor of glucagon secretion. This glucagon-suppression effect is also glucose-concentration dependent and only occurs at glucose concentration above 3.7 mM,[105] and likely mediated through direct effect on pancreatic α cells and also indirect effects through increase in somatostatin and insulin levels from neighboring δ and β cells.[82, 83]

Similar to global *Gipr*$^{-/-}$ mice, global GLP-1R gene knockout (*Glp1r*$^{-/-}$) mice exhibited impaired glucose tolerance and insulin secretion following oral glucose challenge.[108] Following 12 weeks of high fat diet, the impairment of glucose tolerance in *Glp1r*$^{-/-}$ mice was more severe than that of *Gipr*$^{-/-}$ mice.[76] β cell-specific GLP-1R knockdown mice had normal glucose tolerance after oral glucose challenge but the response was blunted by GLP-1R antagonism suggesting a role of extrapancreatic GLP-1R in glucose homeostasis.[109] In addition, double incretin receptor knockout (DIRKO) mice were not impacting insulin levels during oral glucose challenge after 12 weeks of high-fat diet.[76] The complex molecular mechanism of action of GLP-1R has recently been reviewed.[79, 89, 110]

In addition to the effect on the endocrine pancreas, GLP-1 has several extrapancreatic effects. Most notably and unlike GIP, through both central and peripheral actions, GLP-1 decreases appetite leading to decreased food intake and thus body weight. Centrally, GLP-1 promotes satiety through the activation of GLP-1Rs in the hypothalamus and brainstem, which causes a reduction in food intake.[82, 83] GLP-1 has been shown to decrease satiety measures and *ad libitum* food intake in a dose-dependent manner in humans.[111] Peripherally, GLP-1 has pronounced effect on gastric motility and emptying, an effect known as the "ileal brake".[112] The passage of nutrients into the distal small intestine, ileum in particular which is rich in L cells, induces GLP-1 secretion leading to delayed gastric emptying and nutrient absorption and therefore reduced postprandial glucose excursions.[113, 114]

Other extrapancreatic effects of GLP-1 may include increase in hepatic glucose uptake and decrease in hepatic glucose production. In humans, GLP-1 infusion during a pancreatic clamp study resulted in reduction of endogenous glucose production.[115] In dogs, GLP-1 infusion into the portal vein increased non-hepatic glucose uptake without changing insulin and glucagon levels, indicating that a hepatoportal sensor may be regulating the effect of GLP-1 on peripheral glucose metabolism.[116] However, controversy remains over the presence of GLP-1R in adipocytes, skeletal muscle and liver and any possible effects and their underlying mechanisms need further research.[89, 117, 118]

Dual GIPR and GLP-1R activation

Under normal physiology, GIP and GLP-1 work in synchrony to regulate postprandial insulin and glucagon secretion in maintaining glucose homeostasis. However, research has mainly focusing on studying the two hormones separately, especially with the development of GLP-1R agonists in treating type 2 diabetes. GIP had not been used for treating type 2 diabetes because of its lack of insulinotropic effect and possible glucagonotropic action in type 2 diabetes during hyperglycemia.[63,97]

In vitro studies have shown that co-administration of GIP and GLP-1 induced synergistic effect by measuring intracellular cyclic adenosine monophosphate (cAMP) levels in RINm5F insulinoma cells.[119] Incubating isolated human pancreatic islets (both nondiabetic and diabetic human islets) with GIP alone induced greater glucose-stimulated insulin secretion than with GLP-1 alone, and combination of both GIP and GLP-1 provided some additive effect on insulin secretion.[120] GIP and GLP-1 co-infusion studies in rodents yield mixed results in terms of glucose regulation or body weight when compared to GIP or GLP-1 alone.[121] In healthy humans, acute co-infusion of GIP and GLP-1 has synergistic effect on insulin secretion when compared with GIP and GLP-1 alone.[122, 123] However, acute co-infusion of GIP and GLP-1 in patients with type 2 diabetes did not show any added insulinotropic effect to that of GLP-1 infusion alone.[124] Recently, there are significant interests in developing unimolecular dual agonist of GIPR and GLP-1R (dual incretin receptor) with activity at each constitutive receptor.[125-127] This dual incretin receptor concept was recently reviewed.[121]

Incretin hormones in obesity and diabetes

Obesity

What happens to incretins physiology in disease state such as obesity and diabetes? GIP levels are elevated during both fasting and after oral glucose challenged in obesity.[56] Studies showed that K-cell numbers are increased in small intestine of obese *ob/ob* mice compared to lean control mice.[128, 129] When *ob/ob* mice were chronically fed a high-fat diet, the density of K-cells in the small intestine increased by 54% compared to control diet.[130] The increased K-cell density and circulating GIP levels found in obesity might be the result of chronically increased stimuli from the gut lumen, such as an increase in gut endocannabinoid tone. Notably, rodents fed a diet high in linoleic acid, which promoted weight gain, were found to have increased endocannabinoid levels in liver and gut.[131] . In humans, plasma endocannabinoid levels are positively associated with GIP levels, although gut-specific endocannabinoid levels were not assessed.[132] In healthy lean individuals, a cannabinoid

receptor agonist, nabilone, exerted tonic control over non-stimulated GIP secretion because fasting GIP levels in the circulation were increased by 80%.[56]

Disruption of GIP signaling using different animal models—G*ipr*$^{-/-}$ mice, GIP/DT mice lacking GIP-producing cells, treatment with Pro3-GIP (a GIPR antagonist), and vaccination against GIP—appears to prevent high-fat diet-induced obesity and insulin resistance. [75, 133-136] It is clear that dietary fat is a potent stimulus to GIP secretion and GIP plasma levels are increased in obese individuals.[56, 137, 138] Whether there is a causal link between increased GIP signaling and obesity in humans is not clear, however. As mentioned earlier, GIPR gene expression in human adipocytes was negatively correlated with adiposity.[78]

There is no difference in fasting GLP-1 levels between lean and obese healthy subjects but GLP-1 secretion is reduced is response to an oral glucose challenge[56] and there are reports of reduced circulating GLP-1 levels after a meal.[139-141] The lowered GLP-1 response to luminal nutrients in obesity may also be attributed to elevated GIP and endocannabinoid tone as shown in a recent study where increased GIP secretion due to a CBR agonist was associated with significantly reduced GLP-1 release during a glucose challenge.[56]. Furthermore, exogenous GIP infusion at high doses led to a diminution in GLP-1 secretion during a mixed-meal challenge in obese subjects with type 2 diabetes.[63] These new findings support the conclusion that incretins are influenced by endocannabinoids.

Type 2 diabetes

Since the discovery of incretins, the status of GLP-1 and GIP secretion in response to nutrients in type 2 diabetes have been marred by inconsistent findings and conflicting data. Earlier studies reported a slight increase in GIP secretion and reduced GLP-1 secretion in type 2 diabetes.[62, 142, 143] It appears that duration and state of glucose control is an important factor in incretin secretion. In newly diagnosed type 2 diabetes mellitus with relatively good glycemic control (Hemoglobin A1c [A1c] ~6.9%), both GIP and GLP-1 secretion in response to oral glucose and mixed meal challenges are similar or slightly increased when compared with healthy subjects.[20, 92] However, in longstanding type 2 diabetes with poor glycemic control (A1c ~ 8–9%), the GLP-1 response is decreased, whereas GIP secretion is unchanged.[62, 143, 144] Recent meta-analysis of 23 trials with 28 different stimulation tests concluded that GIP secretion in response to glucose and meals is preserved in type 2 diabetes with two caveats: high BMI is associated with increased GIP levels while aging and higher A1c are associated with reduced GIP response.[145] Similar meta-analysis was done in 22 trials for GLP-1

secretion, with 29 different stimulation tests showed that type 2 diabetes in general is not associated with reduced GLP-1 secretion except on a background of poor glycemic control.[146]

In patients with type 2 diabetes, insulin secretion by oral glucose is no longer substantially greater than the response to intravenous glucose.[147] GIP and GLP-1 secretion appear not to play a causal role in this defect as their secretion has been noted to be preserved in type 2 diabetes as stated above. GIP has a more significant contribution to insulin secretion over GLP-1 in healthy humans.[148] In type 2 diabetes, pancreatic islets remain responsive to GLP-1 but are no longer responsive to GIP. Insulin response to exogenous GLP-1 is 3- to 5-fold lower in type 2 diabetes; however, acute GLP-1 administration is able to increase insulin secretion to normal levels and to lower plasma glucose effectively.[123, 149] As elimination of GLP-1 is unchanged, the reason for the reduced incretin effect in type 2 diabetes can be explained in part by reduced β-cell sensitivity to GLP-1 in addition to loss of insulinotropic activity of GIP.[150] Exogenous GIP, even at supraphysiological doses, has markedly reduced insulinotropic actions with little or no glucose-lowering effects in type 2 diabetes, and the metabolic consequence is compounded by increased glucagon secretion during mixed meals and hyperglycemic clamps.[63, 97, 123] Animal studies suggest that exogenous GLP-1 has the ability to increase islet size, enhance β-cell proliferation, inhibit β-cell apoptosis, and regulate islet growth, at least in young rodents.[106, 107, 151] These effects have tremendous implication in the treatment of type 2 diabetes because they directly address one of the fundamental defects in type 2 diabetes, i.e., β-cell failure.

Bariatric surgeries and incretins

Several bariatric surgical techniques are designed to promote weight loss and bring about remission of type 2 diabetes. A meta-analysis of 136 studies included 22,094 patients (1846 patients were in studies with reports of diabetes resolution) who underwent various bariatric surgeries for treatment of morbid obesity and followed for 1–3 years. Within studies showing resolution of diabetes after bariatric surgery, the rate of diabetes resolution for laparoscopic adjustable gastric banding, vertical banded gastroplasty, Roux-en-Y gastric bypass, and bilio-pancreatic diversion were 48%, 68%, 84% and 98%, respectively.[152] Interestingly, another meta-analysis included 94,579 patients (4944 with type 2 diabetes) showed remission rates were equivalent in patients with BMI < 35 kg/m^2 and patients with mean baseline BMI ≥ 35 kg/m^2, 72% versus 71% respectively.[153] Eleven recent randomized controlled trials compared bariatric surgery versus medical management of type 2 diabetes in nearly 800 patients with follow-up duration of 1–5 years, and bariatric surgery achieved superior diabetes remission rate (33–90%) compared to medical management (0–23%).[154]

Diabetes remission after surgical manipulations of the gastrointestinal tract, Roux-en-Y gastric bypass or biliopancreatic diversion procedure, is often observed within days after surgery, even before significant weight loss occurs; whereas with gastric banding, a restrictive procedure involving placing an adjustable gastric band fitted around the stomach near the esophageal junction, diabetes remission might not occur for several months.[155, 156] The physiological and molecular mechanisms underlying the beneficial glycemic effects of bariatric surgery are complex, involve altered endocrine signaling that result from surgical manipulation of the gastrointestinal tract, and are still not completely understood.[156] Pories and colleagues were the first to suggest that incretins might play a role in rapid diabetes remission after gastric bypass.[157] It seems evident that bypassing the upper small intestine and excluding it from contact with nutrients would result in alteration in GIP and/or GLP-1 secretion. Indeed, after gastric bypass or biliopancreatic diversion surgery, post-prandial GIP levels decrease, while GLP-1 levels increase, attributed to rapid gastric emptying and/or direct accelerated delivery of nutrients to the L-cell-rich distal intestine.[158-162] Although increased GIP levels are associated with obesity,[56, 163] it is not known whether decreased GIP secretion is related to diabetes remission in gastric bypass surgery because some studies reported improvement in glucose homeostasis with increase in plasma GIP levels (1 month after surgery) or no change in plasma GIP levels (6 months after surgery) after gastric bypass surgery.[164, 165] Post-prandial GLP-1 levels, however, are markedly elevated after Roux-en-Y gastric bypass and biliopancreatic diversion, and vertical sleeve gastrectomy.[156] Hypersecretion of GLP-1 occurs early in the first six months post-surgery and, in one report, normalized by 12 to 15 months.[166] The hypersecretion of GLP-1 post-surgery is critical for the improvement in β-cell function and glucose homeostasis as demonstrated by in the infusion of exendin-4 (9–39), a GLP-1R antagonist, which reversed this effect.[167] For patients who achieved sustained diabetes remission for greater than 2 years, other factors are likely involved given that GLP-1 secretion returns to normal after approximately one year and administration of exendin-4 (9–39) only marginally impaired post-prandial glucose homeostasis despite decreases in insulin secretion.[168] Comprehensive reviews of the role of gut hormones after bariatric surgery are available.[169, 170]

Clinical application of GIP and GLP-1 in obesity and diabetes

Glucose-dependent insulinotropic polypeptide

Given the strong, glucose-dependent insulinotropic effect of incretins, their therapeutic potentials for diabetes treatment has been vigorously pursued since their discovery. Although GIP has similar insulinotropic action to that of GLP-1, it soon became clear that GIP lacks insulinotropic and glucose-

lowering effects in patients with type 2 diabetes.[97, 147, 171, 172] Exogenous GLP-1 but not GIP administration augmented insulin secretion in patients with type 2 diabetes.[123, 173] Furthermore, GIP have been shown to be elevated in obese individuals, as mentioned above, and to have obesogenic effect, at least in animal models.[56, 163, 174, 175]

In type 2 diabetes, β cells develop resistance to GIP and this GIP resistance might be improved by reducing hyperglycemia. In the VDF Zucker rat, an animal model of type 2 diabetes, GIPR mRNA and protein levels were found to be down-regulated in the presence of hyperglycemia; and GIPR mRNA and protein levels, hence β-cell sensitivity to GIP, were restored when high blood glucose levels were lowered with phloridzin.[176] In patients with type 2 diabetes, one-month treatment with glyburide reduced blood glucose levels and increased GIP sensitivity.[177] A supra-physiological dose of GIP, at five-fold higher than normally observed post-meal, was shown to have a short-lived insulinotropic effect in type 2 diabetic patients, but this increase in insulin did not translate to lowering blood glucose levels as there was a concomitant glucagonotropic effect on α cells.[63] GIP was reported to increase glucagon secretion from the isolated perfused rat pancreas.[70] Further elucidation of the mechanism of GIP resistance and glucagonotropic effect of GIP in patients with type 2 diabetes may present a caveat to GIP as a therapeutic agent. Furthermore, with elevated GIP levels in obesity and effect of GIP in promoting fat storage in adipocytes, blocking GIP signaling has been proposed as a treatment for obesity.[163, 174] Animal studies have shown promising results: in *ob/ob* mice, treatment with Pro^3-GIP (GIPR antagonist) prevented development of diabetes and related metabolic abnormalities;[134, 135] vaccinating C57BL/6 mice with antibodies against GIP reduced body weight gain despite the animals being fed a high-fat diet;[136] genetically deleting GIPR or targeting K-cell ablation in mice both protected against obesity and associated metabolic dysregulation during a high-fat diet.[75, 133] However, GIP antagonism might not be the best route forward because even though it appears to be effective in treating and preventing obesity in animal models, GIP antagonism also reduces glucose-induced insulin secretion in non-diabetic conditions.[178-180] Research is currently being pursued in engineering GIP analogs that would selectively improve β-cell function but have reduced adipogenic and glucagonotropic actions. For example, specially engineered GIP analogs, such as D-Ala^2-GIP_{1-30}, demonstrated equivalent potency to GIP_{1-42} in terms of β-cell function and survival but greatly reduced lipogenic actions.[181] The development of GIPR antagonists has recently been reviewed.[182]

Glucagon-like peptide-1

In type 2 diabetes, exogenous GLP-1 administration increases insulin secretion and lowers plasma glucose effectively even though insulin response is 3- to 5-fold lower when compared to healthy individuals.[97, 123, 149] Furthermore, continuous intravenously administered GLP-1 completely normalized plasma levels of glucose in patients with type 2 diabetes.[183] At pharmacological doses, GLP-1 also has other non-insulinotropic effects: suppressing glucagon secretion in the presence of hyperglycemia and euglycemia, but not hypoglycemia, leading to improved hepatic insulin resistance and glycemic control;[105, 184] slowing of gastric emptying and gut motility, causing delayed nutrient absorption and dampening postprandial glucose excursion;[185] and increasing the duration of postprandial satiety, leading to reduced food intake, weight loss, and improved insulin resistance;[111, 186, 187] all of which formed the foundation of GLP-1-based treatment of type 2 diabetes.

GLP-1R agonists in type 2 diabetes. One major drawback of using native GLP-1 in treating diabetes is its short half-life of about 2 min due to DPP4 activity, as discussed above. After removal of histidine and alanine from the N-terminus, GLP-1 is further hydrolyzed by neutral endopeptidases (NEP) 24.11 at six different places.[188] Due to its biological short half-life, bolus subcutaneous injections of GLP-1 resulted in only a transient effect on insulin secretion and plasma glucose levels.[189] Several approaches have been used to develop GLP-1R agonists to circumvent degradation of GLP-1 by DPP4. GLP-1R agonists can be classified as short-acting or long-acting compounds based on their pharmacokinetics profile – whether they provide intermittent or continuous activation of GLP-1Rs, respectively. Seven GLP-1R agonists, with half-lives ranging from 2.4 to 165 hrs, were approved by the US Food and Drug Administration (FDA) for use in treating type 2 diabetes. Exenatide (twice daily) and lixisenatide are the two short-acting GLP-1R agonists; exenatide (once weekly), liraglutide, albiglutide, dulaglutide, and semaglutide are the five long-acting GLP-1R agonists. Albiglutide is currently discontinued because of low volume sales.[190] Two GLP-1Rs with different mode of delivery—oral route or via implantable, subdermal, osmotic titanium mini-pump—are currently awaiting FDA approval.

Short-acting GLP-1R agonists. The first strategy was the use of exendin-4, a 39-amino acid peptide produced in the salivary glands of Gila monster (*Heloderma suspectum*) with 53% amino acid homology to full-length GLP-1. Exendin-4 is not a substrate for DPP4 because it has a Gly^8 in place of an Ala^8. It also lacks some of the target bonds for NEP, and its secondary and tertiary structures may also prevent NEP hydrolysis. Exenatide, the biosynthetic version of exendin-4, must be injected subcutaneously. It is renally cleared through glomerular filtration, has a terminal half-life of about 2.4 h, has biological effects up to 8 h after dosing, and is still detectable in the plasma 15 h after one

subcutaneous injection.[191-193] It needs to be dosed twice a day, however, to maintain glucose-lowering effects. To increase the half-life of exenatide, lixisenatide was developed by deleting Pro^{38} and adding six terminal lysine residues. This modification increased the half-life of native exenatide to about 3 hours and twice daily administration is not advised.[194, 195]

In the 24-week GetGoal-X trial, the efficacy and safety of exenatide twice daily versus lixisenatide as an add-on to metformin for treating type 2 diabetes were compared.[196] Lixisenatide lowered A1c by 0.79% compared to 0.96% for exenatide twice daily and reached the predefined noninferiority margin criteria of 0.4%. Weight reduction was less in the lixisenatide group compared to the exenatide twice daily group (–2.96 kg vs. –3.98 kg). Patients treated with lixisenatide had fewer gastrointestinal adverse effects (43.1% vs. 50.6%) and experienced fewer episodes of symptomatic hypoglycemia (2.5% vs. 7.9%).

*Long-acting GLP-1R agonists***.** Several approaches have been used to increase the half-life of native GLP-1 peptide: modifying the native GLP-1 amino acid sequence to prevent DPP4 degradation; utilizing a fatty acid chain to delay absorption from subcutaneous tissue after injection; or using protein binding to prevent renal elimination. Four of the FDA-approved GLP-1R agonists are derived from native GLP-1 peptide that use one or more of these approaches: liraglutide, albiglutide, dulaglutide, and semaglutide. The fifth GLP-1R agonist is extended release exenatide (once weekly).

Liraglutide is a long-acting GLP-1R agonist with a substitution of Lys^{34} with Arg^{34} and an attachment of a C-16 free-fatty acid derivative via a glutamoyl spacer to Lys^{26}. The hydrophobic properties of the free-fatty acid derivative result in heptamer formation and delayed absorption from subcutaneous injection sites. It also enabled formation of noncovalent binding of liraglutide to albumin; therefore, increasing plasma half-life by preventing renal clearance. After subcutaneous injection, maximum plasma concentrations of liraglutide are reached after 10–14 h, and it has a half-life of 11–13 hours.[197, 198]

Albiglutide is generated by the genetic fusion of two sequential copies of DPP4-resistant GLP-1 with human albumin.[199] Modification is made to the amino acid sequence of native GLP-1 at position 8 (substitution of Ala^{8} with Gly^{8}) to protect it from DPP4 hydrolysis.[199] This intrinsic design significantly increased the half-life of albiglutide to about 5 days and it can be administered once weekly.[200, 201]

Dulaglutide consists of two DPP4-resistant GLP-1 analogues that have been covalently linked to a constant fragment (Fc) of a human immunoglobulin class 4 (IgG4). The size of ensuring compound, at 59.7 kDa, reduces its renal clearance. Furthermore, the amino acid sequence of the GLP-1 analog has been modified at 3 positions—substitution of Ala^{8} with Gly^{8}, Gly^{22} to Glu^{22}, and

Arg[36] to Gly[36]—to prevent DPP-4 hydrolysis.[202] Similar to albiglutide, these modifications extended the half-life of dulaglutide to about 5 days allowing for once weekly administration.[203]

The synthesis of semaglutide was based on liraglutide. Semaglutide has two amino acid substitutions compared to native GLP-1 (Ala[8] by Aib[8] and Lys[34] by Arg[34]), and similar to liraglutide, is attached at Lys[26] with a longer linker and a longer fatty acid chain of C18 instead of C16.[204] These modifications increase the half-life of semaglutide to about 7 days.[205] The current FDA-approved semaglutide is administered via subcutaneous injection. An oral form of semaglutide is under development, where semaglutide is co-formulated with the absorption enhancer sodium N-(8-[2-hydroxybenzoyl] amino) caprylate (SNAC) to facilitate its absorption across the gastric epithelium.[206] Similar to injectable semaglutide, oral semaglutide has a half-life of about 7 days, and an FDA New Drug Application was submitted in March 2019.[206, 207]

The fifth long-acting GLP-1R agonist is a sustained-release formulation of exenatide consisting of injectable microspheres of exenatide and poly (D,L lactic-co-glycolic acid), a common biodegradable medical polymer with established use in absorbable sutures and extended release pharmaceuticals, that allows gradual drug delivery at a controlled rate.[208, 209]

GLP-1R agonist via infusion pump. ITCA 650 is a drug-device combination product in which a continuous subcutaneous delivery of exenatide can be achieved for up to 12 months using a titanium matchstick-sized osmotic mini-pump placed in the subdermis of the abdominal wall.[210] A recently published phase 3 double-blind, placebo-controlled trial demonstrated that ITCA delivery of exenatide significantly reduced A1c (–1.2%) and weight in type 2 diabetic patients already taking oral glucose-lowering agents.[211] A phase 3 open-label trial in type 2 diabetic patients with baseline A1c of 10.8% showed that after 39 weeks of treatment, a reduction of A1c of –2.8% was achieved.[212]

Head-to-head comparison trials of GLP-1R agonists. As a drug class for type 2 diabetes, the GLP-1R agonists have proven efficacy for lowering A1c and body weight together with a reduced risk of hypoglycemia compared with insulin or sulphonylureas.[213] To date, the results from eleven phase III randomized trials that directly compare different pairs of FDA-approved GLP-1RAs have been published: DURATION-1 (exenatide twice daily versus exenatide once weekly)[214]; LEAD-6 (exenatide twice daily versus liraglutide once daily)[215]; DURATION-5 (exenatide twice daily versus exenatide once weekly)[216]; GetGoal-X (exenatide twice daily versus lixisenatide once daily)[196]; exenatide (twice daily versus weekly)[217]; DURATION-6 (liraglutide once daily versus exenatide once weekly)[218]; HARMONY-7 (liraglutide once daily versus albiglutide once weekly)[219]; AWARD-1 (exenatide twice daily versus dulaglutide once weekly)[220]; AWARD-6 (liraglutide once daily versus dulaglutide once weekly)[221]; SUSTAIN-3 (exenatide once weekly versus semaglutide once weekly)[222]; SUSTAIN-7

(dulaglutide once weekly versus semaglutide once weekly)[223]. Patients included in these studies were treated with various oral glucose-lowering agents or diet and exercise prior to enrollment.

*A1c reduction***.** All GLP-1R agonists, short- or long-acting, have demonstrated robust reductions in A1c, with reduction ranging from 0.8–1.9% in phase III clinical trials. [196, 214-223] In head-to-head comparison of GLP-1R agonists, long-acting GLP-1R agonists have generally proven superior to exenatide twice daily with significantly greater reduction in A1c levels: DURATION-1 (exenatide twice daily [–1.5%] versus exenatide once weekly [–1.9%]); LEAD-6 (exenatide twice daily [–0.8%] versus liraglutide once daily [-1.1%]); DURATION-5 (exenatide twice daily [–0.9%] versus exenatide once weekly [–1.6%]); Exenatide (exenatide twice daily [–1.1%] versus exenatide once weekly [–1.4%]); AWARD-1 (exenatide twice daily [-0.8%] versus dulaglutide once weekly [–1.4%]). [214-217, 220] It is important to note that results are not comparable across studies because of differences in study design and patient cohorts. Even though lixisenatide has not yet been compared directly with a long-acting GLP-1R agonist in a phase 3 clinical trial, lixisenatide lowered HbA1c by 0.79% compared to 0.96% for exenatide twice daily in the GetGoal-X trial, which was a statistically significant difference.[196]

Post-prandial glucose excursion profile. Short-acting and long-acting GLP-1R agonists have differential effects on fasting and postprandial glucose due to their pharmacology. Similar to native GLP-1, short-acting GLP-1R agonists provide intermittent stimulation of GLP-1R and preserve their ability to delay gastric emptying. This delay in gastric emptying, together with suppression of inappropriate glucagon secretion, results in markedly lower post-prandial glucose excursion after short-acting GLP-1R agonists administration.[224-227] With higher postprandial glucose excursion, long-acting GLP-1R agonists induce an increase in postprandial insulin concentrations,[228] whereas short-acting GLP-1R agonists may actually lead to a decrease.[224] Long-acting GLP-1R agonists provide a continuous exposure to GLP-1Rs and this seems to cause downregulation of the effects on gastric emptying that, in turn, does not reduce postprandial glucose excursions to the same extend as do short-acting GLP-1R agonists.[214, 227] Hence, long-acting GLP-1R agonists has less reduction in post-prandial glucose but allow enhanced effects on the whole 24-h glucose levels, ultimately resulting in superior effects on lowering fasting plasma glucose and A1c.[229] Long-acting GLP-1R agonists provide better glycemic control than short-acting GLP‑1R agonists because their use results in higher insulin levels in the fasting state resulting in better suppression of gluconeogenesis in type 2 diabetic patients.[214, 215, 218]

Antibodies formation. Antibody formation to exenatide is frequent after treatment and is generally of no clinical relevance. A post-hoc analysis of 21 exenatide trials of various duration with over 4000 patients showed that the formation of anti-exenatide antibodies is common (37% in

exenatide twice daily; 57% in exenatide weekly), mostly of low titer, and peak early at 24-30 weeks, and have no apparent effect of efficacy. The titers subsequently declined (exenatide twice daily: 25 % at 52 weeks and 17% at 3 years; exenatide weekly: 45% at 52 weeks). There was, however, a small subgroup of patients where high anti-exenatide antibody titer was associated with smaller reduction in A1c.[163] For lixisenatide treatment, 56-60% of patients developed anti-lixisenatide antibodies after starting treatment and development of anti-lixisenatide antibodies also appear to be of little clinical relevance.[197] The GLP-1R agonists based on native GLP-1 peptide generally have much lower antibody responses (8–9% for liraglutide; 3–7% for albiglutide; 1–3% for dulaglutide; 1.7% for semaglutide), and these antibodies do not lead to a clinically relevant effect on glycemic control.[171, 198-200]

Weight reduction. GLP-1 has a well-documented effect on satiety.[82, 83] As a drug class, GLP-1R agonists lead to a significantly greater effect on weight reduction than most other antidiabetic drug classes, with a weighted mean difference of –2.9 kg (CI: –3.6 to –2.2) in a meta-analyses of 25 randomized controlled trials using exenatide twice daily, exenatide once weekly or liraglutide once daily.[230] Within the GLP-1R agonist drug class, long-acting GLP-1R agonists have greater effect on fasting plasma glucose and A1c reduction while short-acting GLP-1R agonists work best at suppressing post-prandial glucose excursions. There is, however, no between-class difference in body weight reduction in head-to-head comparison trials of GLP-1R agonists.[190] For example, exenatide twice daily lead to similar weight reductions as did exenatide extended release (once weekly), liraglutide and dulaglutide.[215, 216, 220, 231] Newer compounds, such as semaglutide (1.0 mg once weekly), have shown significant average weight loss of up to 6.5 kg after 40 weeks of treatment.[223]

Cardiovascular outcome trials (CVOTs). Since 2008, FDA and later EMA added cardiovascular safety as a required outcome for approval of new glucose-lowering treatments for type 2 diabetes.[232, 233] CVOT was not included in the clinical trial for exenatide twice daily as it was approved before 2008. For short-acting GLP-1R agonists, ELIXA trial (lixisenatide versus placebo) showed non-inferiority in composite cardiovascular endpoint when compared to placebo in type 2 diabetic patients with acute coronary syndrome or unstable angina.[234] CVOTs for long-acting GLP-1R agonists published to date demonstrated either non-inferiority or superiority composite cardiovascular endpoint in patients with type 2 diabetes and established cardiovascular disease or high cardiovascular risk. LEADER trial (liraglutide versus placebo) reported cardiovascular benefit in composite cardiovascular endpoint (HR 0.87; 95% CI 0.78-0.97; *P* = 0.01 for superiority).[235] SUSTAIN-6 trial (semaglutide versus placebo) and HARMONY trial (albiglutide versus placebo) also reported similar results (HR 0.74; 95% CI 058-0.95; *P* = 0.02 for superiority) and (HR 0.78; 95% CI 0.68-0.90; *P* =

0·0006 for superiority), respectively [236, 237] Both EXSCEL (exenatide once weekly versus placebo) and PIONEER-6 (oral semaglutide versus placebo) showed non-inferiority in composite cardiovascular endpoint (HR 0.91; 95% CI 0.83-1.00; *P* = 0.06 for non-inferiority) for EXSCEL and (HR 0.79; 95% CI 0.57-1.11; *P* < 0.001 for non-inferiority) for PIONEER-6.[238, 239] Given these results with some GLP-1R agonists demonstrating beneficial cardiovascular effect while others not, studies are needed to delineate the underlying mechanisms by which these GLP-1R agonists might affect cardiovascular risk. A review of CVOTs in type 2 diabetes was recently published.[240]

GLP-1R agonists in treating obesity. The ability of GLP-1R agonists to induce weight loss as discussed above, is now well established by clinical trials designed for managing type 2 diabetes; however, the magnitude of weight loss varies among compounds. Currently, liraglutide 3 mg once daily is the only GLP-1R agonist approved by the FDA for treating obesity. A series of randomized clinical trials evaluated the efficacy and safety of liraglutide 3 mg daily for weight management in over 4000 patients without diabetes and who had a BMI of at least 30 kg/m^2 or at least 27 kg/m^2 with comorbidities.[241-243] With diet and exercise, compared with placebo, liraglutide 3 mg once daily provided an additional weight reduction of 4.2–5.4% of body weight after 56 weeks.[242, 243] In a 52-week phase 2 trial of 957 individuals without diabetes and with BMI of at least 30 kg/m^2, patients were randomized to receive semaglutide (0.05 mg, 0.1 mg, 0.2 mg, 0.3 mg, or 0.4 mg daily), liraglutide (3 mg daily) or placebo, in combination with diet and exercise. Semaglutide 0.05–0.4 mg per day resulted in dose-dependent weight losses over 52 weeks that were significantly greater than placebo at all doses, and higher than liraglutide (3 mg daily) at doses of 0.2 mg per day or more.[244] Semaglutide (0.05 mg daily) and semaglutide (0.4 mg daily) provided a weight reduction of 6.8% and 16.2% respectively, in comparison to liraglutide (3 mg daily) of 8.3% and placebo of 2.3%.[244]

GLP-1R agonists in treating prediabetes. The use of GLP-1R agonists in preventing diabetes has so far been investigated only with liraglutide. In a 56-week randomized controlled trial involving 3731 overweight or obese patients without diabetes, 2487 were randomized to liraglutide 3mg daily and 1244 to placebo.[242] At baseline 61.2% of the patients had prediabetes. After 56 weeks, the prevalence of prediabetes was significantly lower in the liraglutide group compared to the placebo group, and type 2 diabetes developed in more patients in the placebo group.[242] From 56 weeks on, patients with prediabetes at screening continued on treatment (liraglutide 3 mg daily or placebo) for another 2 years.[245] At the end of 2 years, 2% in the liraglutide group developed diabetes compared to 6% in the placebo group. In addition, 66% of individuals in the liraglutide group regressed back to

normal glucose tolerance while only 36% of those did so in the placebo group.[245] Currently, none of the GLP-1R agonists are approved by FDA for the treatment of prediabetes.

Safety Issues and tolerability. *Antibodies formation*. Antibody formation to exenatide is frequently reported after treatment and is generally of no clinical relevance. A post-hoc analysis of 21 exenatide trials of various durations with over 4000 patients showed that the formation of anti-exenatide antibodies is common (37% in exenatide twice daily; 57% in exenatide weekly), mostly of low titer, and peak early at 24–30 weeks, and have no apparent effect of efficacy. The titers subsequently declined (exenatide twice daily: 25 % at 52 weeks and 17% at 3 years; exenatide weekly: 45% at 52 weeks). There was, however, a small subgroup of patients where high anti-exenatide antibody titer that was associated with smaller reduction in A1c.[193] For lixisenatide treatment, 56–60% of patients developed anti-lixisenatide antibodies after starting treatment and development of anti-lixisenatide antibodies also appear to be of little clinical relevance.[246] The GLP-1R agonists based on the native GLP-1 peptide generally have much lower antibody responses (8–9% for liraglutide; 3–7% for albiglutide; 1–3% for dulaglutide; 1.7% for semaglutide), and again, these antibodies do not lead to a clinically relevant effect on glycemic control.[201, 247-249]

Gastrointestinal side effects. Gastrointestinal adverse effects are common in patients treated with GLP-1R agonists, with nausea, vomiting or diarrhea being the most frequently reported.[250] A systematic analysis of 32 published phase 3 clinical trials on GLP-1R agonists with 10,367 patients found the following: (1) risk of nausea and diarrhea was dose-dependent for long-acting GLP-1R agonists; (2) nausea and vomiting were more common with metformin combination therapy; (3) compared to exenatide twice daily, lixisenatide treatment was associated with less nausea and diarrhea; (4) compared to liraglutide, exenatide weekly and albiglutide are associated with less nausea and diarrhea; (5) compared to short-acting GLP-1R agonists, long-acting GLP-1R agonists were associated with less nausea and vomiting but more diarrhea.[251]

*Pancreatitis and pancreatic cancer***.** Controversies surrounding a suspected association between pancreatitis or pancreatic cancer with the use of GLP-1R agonists surfaced after its introduction in 2005. A study found a more than 10-fold increase in reported pancreatitis and 2.9-fold increase in reported pancreatic cancer in patients treated with exenatide compared to other therapies using the FDA Adverse Event Reporting System (FAERS) database.[252] However, the FAERS is used for reporting adverse events and is subjected to bias.[253] To address this concern, both the FDA and European Medicines Agency (EMA) reviewed the preclinical and clinical studies regarding the risk of pancreatitis and pancreatic cancer and concluded that the data were inconsistent with a causal association between GLP-1R agonists and pancreatic adverse events.[254] A meta-analysis of

three randomized placebo-controlled trials of at least 24 months duration, involving 9347 patients on GLP-1R agonist and 9353 on placebo found no evidence of increased risk of pancreatitis.[255] A retrospective cohort study involving almost 1 million patients initiating antidiabetic medications also showed no increased risk of pancreatic cancer with GLP-1R agonists use.[256] Therefore, it is safe to conclude that GLP-1R agonists are unlikely to cause either pancreatitis or pancreatic cancer.

Current clinical guidelines. In the latest Consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) published in October 2018, GLP-1R agonists have been recommended as part of glycemic management for the following: (1) patients with established atherosclerotic cardiovascular disease (specifically GLP-1R agonists with proven cardiovascular benefit); (2) patients with need to minimize hypoglycemia; and (3) patients with need to minimize weight gain or promote weight loss.[257]

Dual incretin receptor agonists. Recently, there has been a surge in interest in developing uni-molecular dual agonist of GIPR and GLP-1R with activity at both incretin receptors for even greater glucose lowering effects than seen with GLP-1R agonists alone.[125-127] A randomized, placebo-controlled and active comparator-controlled phase 2 trial was reported for one of these dual incretin receptor agonists LY3298176 in patients with type 2 diabetes. After 26 weeks, LY3298176 showed superior A1c control with greater weight loss and acceptable tolerability profile, compared with dulaglutide.[258] There are at least 3 other dual incretin receptor agonists in various stages of development: NNC0090-2746 (also known as RG7697), DA-JC1 and DA3-CH.[259-261]

Evolving understanding of the enteroendocrine cell biology, gut microbiota, and endocannabinoid system

Classical view of enteroendocrine cells designates one hormone production per cell and these cells were named accordingly; i.e., K cell secretes GIP and L cell secretes GLP-1. This uni-hormonal phenotype was largely based on anatomical appearances and histochemical and staining characteristics.[262] Over the years, researchers have shown that the enteroendocrine system is much more complex with heterogenous enteroendocrine cell-types and the knowledge that one enteroendocrine cell may secrete two or more different hormones instead of the traditional concept of one cell-one hormone. Reports showed colocalization of GIP and GLP-1 in enteroendocrine cells called K/L cells that accounted for about 40–50% of the K, L and K/L enteroendocrine cells in the duodenum and up to 55–75% in the mid-small intestine.[20, 38] As many as six enteroendocrine hormones (CCK, GLP-1, GIP, PYY, neurotensin, and secretin) have been found in the same enteroendocrine cell.[263, 264] Our group has even found the presence of hormones such as ghrelin,

insulin and GLP-1 in one taste cell type in taste buds in the tongue.[265-267] Furthermore, recent findings that enteroendocrine cells can actually switch hormone expression depending on local cues and tissue compartments will surely revolutionize the field of gut endocrinology.[268]

The local environment that enteroendocrine cells interacts with includes, but is not limited to gut microbiota, ingested food and other secreted acids and bile acids. The gut microbiota has been shown to influence a whole host of human physiology such as nutrient absorption, immune function, metabolic and endocrine functions.[269, 270] Regulation of enteroendocrine hormone secretion by gut microbiota is best shown by studies in germ-free mice. Germ-free mice were found to have higher circulating GLP-1 levels and increased pro-glucagon gene expression and GLP-1 immuno-positive cell density in the distal gastrointestinal tract. Introduction of gut microbiota from conventionally raised mice to these germ-free mice normalized pro-glucagon gene expression and GLP-1 immuno-positive cell density as well as circulating GLP-1 levels.[271]

Gut microbiota convert dietary polysaccharides that cannot be digested by the host into short-chain fatty acids (SCFAs).[272] SCFAs has been shown to increase the number of L cells in mouse and human intestinal epithelium (*in vitro*) through increasing expression of transcription factor neuronal differentiation 1 (*Neurod1*); thus, resulting in increased GLP-1 secretion.[273] Blocking the NOTCH signaling pathway with dibenzoazepine, a γ-secretase inhibitor, led to elevated expression of Neurod1 and subsequent increase in K and L cell numbers accompanied by increased GIP and GLP-1 secretion.[274] Therefore compounds that alter gut microbiota may, in turn, regulate secretion from enteroendocrine cells. Low-calorie sweetener is an example of such compound. Regular consumption of low-calorie sweeteners is associated with greater increases in GIP secretion following nutrient intake in humans.[44] The possible involvement of gut microbiota in modulating the association between low-calorie sweetener consumption with increased GIP secretion can be inferred from rodent data. Long-term feeding of low-calorie sweetener to mice altered gut microbiota to those with over-representation of glycan degradation pathways, leading to increased formation of short-chain fatty acids (SCFAs), which has been shown to increase the number of K and L cells, and incretin secretion.[45]

Another exciting research area that shows great potential is the possible role of the endocannabinoid system in regulating incretin secretion, and how gut microbiota may constitute an integral part of this process. The first study to show that bacteria *Lactobacillus acidophilus* modulates CBR expression was reported to occur in intestinal cells in rats.[275] The possible interactions of the endocannabinoid system with gut microbiota were recently reviewed Cani and colleagues.[269] The role of the endocannabinoid system in regulating incretin secretion in humans was recently reported for the first time.[56] When compared to placebo, nabilone (CBR agonist)

administration to healthy human subjects, as mentioned above, resulted in significantly elevated fasting GIP levels and post-OGTT GIP levels, but no change in fasting GLP-1 levels together with significantly lower post-OGTT GLP-1 levels.[56] The mechanisms weaving together nutrients, gut microbiota, endocannabinoid system and enteroendocrine cells are complex and present an exciting frontier for further research. Dysregulation in the endocannabinoid system and gut dysbiosis has been linked to obesity and diabetes and is an area of active research.[269]

Conclusions

Since the discovery of GIP 50 years ago, tremendous progress has been made in understanding the physiology and pathophysiology of incretins in relations to two of the most pressing global public health crises: obesity and diabetes. With that understanding came the development of a whole new drug class – GLP-1R agonists – for managing type 2 diabetes and obesity. As a drug class, GLP-1R agonists have been shown in phase 3 randomized control trials to be very effective in long-term A1c lowering and lead to weight reduction. Emerging data also showed the benefit of GLP-1R agonists, compared to placebo, in preventing diabetes progression as well as reducing heart attacks, non-fatal strokes, cardiovascular death and all-cause mortality in type 2 diabetes, at least in liraglutide and semaglutide.[235, 236, 239, 276] A recent review by Nauck and colleagues provided a comprehensive review of cardiovascular actions of GLP-1R agonists.[277] In addition, a new class of agents, dual incretin receptor agonists, are currently being developed. One of the most exciting recent findings in which enteroendocrine cells can actually switch hormone expression depending on local cues and tissue compartments adds another dimension to the ever-evolving field of incretins. Understanding the interface and interactions between enteroendocrine cells, the endocannabinoid system, gut microbiota, food intake and its composition is the next frontier in gut endocrinology for the next half century.

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Competing interests

The authors have no competing interests.

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