Review

Incretin Agonism: Sustainable Efficacy or Surreptitious Hazard?

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Incretin Agonism: Sustainable Efficacy or Surreptitious Jeopardy?

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Abstract: Recent clinical trials using synthetic incretin hormones, glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) receptor agonists have demonstrated that these treatments ameliorated many complications related to obesity, emphasizing the significant impact of body weight on overall health. Incretins are enteroendocrine hormones secreted by gut endothelial cells triggered by nutrient ingestion. The phenomenon that oral ingestion of glucose elicited much higher insulin secretion than intravenous injection of equimolar glucose is known as incretin effect. This also alludes to the thesis that food intake is the root cause of insulin resistance. Amylin is co-expressed with insulin from the pancreas β-cells but does not have insulinotropic function. Amylin suppresses glucagon secretion, slowing gastric emptying, and suppressing the central nervous system (CNS) reward center leading to weight loss. However, amylin can self-aggregate and cause serious cytotoxicity and may cause β-cell apoptosis. Glucagon is secreted by the pancreatic α-cells and participates in glucose homeostasis in glucose-dependent manner. In hypoglycemia, glucagon increases blood glucose level by glycogenolysis and gluconeogenesis and inhibits glycogenesis in the liver. Some triple agonists in combination with glucagon and dual incretins are already being developed. These advances bring to the question "Are the benefits of these anti-obesity treatments sustainable?" Chronic agonism may decrease the number of receptors. Also, long-term stimulation may cause β-cell exhaustion and failure. Additionally, instead of endogenous control of the appetite, exogenous control of satiety and food intake may hinder the long-term sustainability of these treatments. We will discuss the incretins' mechanism of action, challenges, and future directions.

Keywords: glucagon-like peptide 1; glucose-dependent insulinotropic polypeptide; satiety; proopiomelanocortin (POMC); neuropeptide Y (NPY); agouti-related peptide (AgRP); GABAergic neurons

Introduction

By the definition of La Barre[1], an incretin should satisfy two criteria: (a) it must be released by nutrients, especially carbohydrates and; (b) it must stimulate insulin secretion in the presence of elevated blood glucose levels. Among the potential incretin candidates such as gastrin, secretin, cholecystokinin (CCK), vasoactive intestinal peptide (VIP), peptide histidine isoleucine (PHI), gastrin-releasing peptide (GRP), entero-glucagon, glucagonlike peptides (GLPs), and glucose-dependent insulinotropic polypeptides (GIP), GLP-1 and GIP are the most important and most researched incretins.

GIP is secreted from K cells in the small intestine by proteolysis of pre-pro-GIP,[2] while GLP-1 is expressed in the intestinal L cells and derived from pro-glucagon through differential proteolytic cleavage.[3] Both GIP and GLP-1 act on G protein-coupled receptors on islet β cells to stimulate insulin secretion. [4] Although both stimulate insulin secretion, there are subtle differences between

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these two molecules. GLP-1 suppresses appetite via neuronal inputs, while GIP's role in appetite is conflicting. Although animal studies report GIP's involvement in appetite suppression.[5–7] and neurons expressing the GIP receptors are identified in the brain of both mice and humans, human studies including randomized controlled trials, reported almost no effects of GIP on appetite suppression. [8–10] We believe that appetite control involves numerous hormones and neurons, so focusing on just one neuron or molecule as was done in most animal studies might not provide a complete picture. Moreover, each molecule can be stimulated or inhibited. Thus, the end results may have many possible outcomes.

The clinical successes of the incretin trials utilizing GLP-1 receptor agonists (GLP-1ras) and GIP receptor agonists (GIPras) have been nothing short of "astonishing" in weight reduction,[11,12] and glucoregulation in type 2 diabetes (T2D)[13] and prediabetes.[14] Further, incretin efficacies are reported in the prevention of cardiovascular complications,[15] and delaying the complication of diabetes-related kidney dysfunction.[16] The most recent trials utilizing dual (combination of GLP-1 and GIP) or triple receptor agonists demonstrated more robust weight reduction and glycemic control than GLP-1 receptor agonists (GLP-ras) alone.[17–21]

We have summarized some key randomized trial results showing these benefits in **Table 1.** These are not a complete collection of references but only essential trials that demonstrated the superiority of incretin agonism compared to traditional diabetes treatments or other GLP-1 ras.

Table 1. Key randomized trial results on incretin agonism and health outcomes.

First author, trial name/ID, year, phase	Sample size, duration	Target population, methods	Objectives, Results (s), & comments
Le Roux, [NCT01272219], 2017, Phase 3[14]	N=2254, 68 weeks or 160 weeks	Prediabetic obese and overweight with co-morbidities cohort Drug: Lira 3.0mg or placebo SC injection once daily	Objectives: Weight reduction /maintenance, T2D onset delay. B Results: y 160 weeks, DM Dx given to (2%) of 1472 in Lira vs. (6%) of 738 in the placebo. Time to DM diagnosis was 99 wks in Lira vs. 87 wks in placebo.
Husain M, PIONEER 6 [NCT02692716], 2019, phase 3[15]	N=3183, Median 62 weeks	T2D with high-cardiovascular risk cohort Drug: Sema or placebo Oral administration once daily	Objectives: Cardiovascular safety of oral Sema, QD in T2D patients. Primary outcome: incidence of MACE Results: MACE occurred in 3.8% in Sema vs. 4.8% in placebo including 15 CVD mort. In Sema Vs. 30 in placebo.
Frías JP, AWARD-11 [NCT03495102], 2021, phase 3[13]	N=1842, 52 weeks (36 weeks primary endpoint)	T2D Patients inadequately controlled with metformin Drug: Dula 1.5mg, 3.0mg or 4.5mg SC injection once weekly	Objectives: Change in HbA_{1c} by week 36 from baseline. Results: At 36wks, Dula 4.5 mg superior to 1.5mg with [ETD] -0.24% but Tx estimand of 3.0 mg was not significant (P = 0.096). However, vomiting nearly doubled in 4.5 mg level. (5.6% vs. 9.3%)
Rubino D, STEP-4 [NCT035489	N=803, 68 weeks	Obese or overweight cohort without T2D Drug: Sema 2.4mg or placebo	Objectives: Comparison of SC Sema continued or switch to placebo both with lifestyle intervention.

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87], 2021, phase 3a[11]		SC injection once weekly Primary outcome: Change in body weight (%)	wt. change week 20- 68: Sema -7.9% vs placebo +6.9%. G-I adverse events: Sema 49.1% vs placebo 26.1% (1.88 times more in Sema group.)
Frías JP, SURPASS-2 [NCT03987919], 2021, phase 3[17]	N=1879, 40 weeks	Metformin-treated T2D cohort Drug: Sema 1mg or Tirzep 5mg, 10mg, 15mg SC injection once weekly	Objectives: Compare effect of Sema and Tirzep on blood sugar levels. Outcome=Change in HbA_{1c} by week 40. The diff. btw groups Tirzep 5-mg, 10-mg, and 15-mg and Sema were -0.15%, −0.39% & −0.45%, respectively. SAE: 5-7% in Tirzep vs. 3% in Sema.
Ludvik B, SURPASS-3 [NCT03882970], 2021, phase 3[22]	N=1444, 52 weeks	Metformin-treated or metformin with SGLT2i-treated T2D cohort Drug: Tirzep 5mg, 10mg, 15mg or insulin degludec 100 U/mL (titrated) SC injection once weekly (Tirzep), SC injection once daily (insulin degludec)	Objectives: Assess safety and efficacy of Tirzep versus insulin degludec on blood sugar levels Results: Non-inferiority of Tirzep to insulin. HbA1c change in Tirzep 5, 10, 15 mg at wk 52 were -1·93%, -2·20%, -2·37%, respectively and -1.34% in insulin. G-I adverse events: 7% in Tirzep vs. 1% in insulin group. Hypoglycemia: 4% in Tirzep vs. 7% in insulin gr.
Del Prato S, SURPASS-4 [NCT03730662], 2021, phase 3[23]	N=2002, 52 weeks (treatment continued until maximum 104 weeks)	Metformin-treated, sulfonylurea-treated, SGLT2i-treated T2D cohort Drug: Tirzep 5mg, 10mg, 15mg or glargine 100 U/mL (titrated) SC once weekly (Tirzep), SC once daily (glargine)	Objective: Assess efficacy and safety of Tirzep versus insulin glargine in adults with high CVD risk and T2D. Primary outcome: Non-inferiority of Tirzep 10mg or/and 15mg versus glargine. Mean HbA_{1c} change at week 52: -2·43% and -2·58%, with Tirzep 10, 15 mg respectively vs.-1·44% with glargine.
Rubino DM, STEP-8 [NCT04074161], 2022, phase 3b[12]	N=338, 68 weeks	Obese or overweight cohort without T2D Drug: Sema 2.4mg or Lira 3.0mg or placebo (matching for both conditions) SC injection once weekly (Sema), SC injection once daily (Lira)	Objectives: Assess the efficacy of once-weekly Sema vs. once-daily Lira on weight loss. Change in body weight (%) by week 68. Mean Wt. change from baseline: -15.8% with Sema, -6.4% with Lira, -1.9% with placebo. G-I adverse events: 84.1% with Sema, 82.7% with Lira.
Wilding J, STEP 1-extension [NCT03548935], 2022[24]	N=327, 1 year after withdrawal from STEP-1	Extension analysis Previous drug: Sema 2.4mg or placebo	Objectives: body weight changes and cardio-metabolic factors following Sema withdrawal. Primary outcome: One year after withdrawal of weekly Sema 2.4

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			mg + lifestyle intervention, participants regained two-thirds of their prior weight loss.
Heerspink H, SURPASS-4 Post Hoc Analysis, 2022[16]	N=2002, Median 85 weeks (104 weeks max)	Metformin-treated, sulfonylurea-treated, SGLT2i-treated T2D cohort Drug: Tirzep 5mg, 10mg, 15mg or glargine 100U/mL (titrated) SC injection once weekly (Tirzep), SC injection once daily (glargine)	Objectives: Compare the effects of Tirzep and insulin glargine on kidney. Primary outcome: tirzepatide slowed the eGFR decline (1.4 vs 3.6 mL/min) and UACR increased with insulin while with Tirzep decreased by -6.8% compared with insulin glargine.
Dahl D, SURPASS-5 [NCT04039503], 2022, phase 3[25]	N= 475, 40 weeks	T2D with titrated insulin glargine on glycemic control cohort Drug: Tirzep 5mg, 10mg, 15mg or placebo SC injection once weekly	Objectives: Assess efficacy and safety of Tirzep in T2D patients receiving inadequate glycemic control. Primary outcome: Mean changew in HbA_{1c} were −2.40% , −2.34%, and −0.86% with 10mg, 15-mg Tirzep and placebo, respectively.
Lincoff AM, SELECT [NCT03574597], 2023, phase 3[26]	N=17604, Mean 137 weeks (Mean follow up 160 weeks)	Obese or overweight cohort with CVD and without T2D Drug: Sema 2.4mg or placebo SC injection once weekly	Objectives: Assess reduction in risk of having cardiovascular events. Primary outcome= MACE (CVD mortality+ nonfatal MI+ nonfatal stroke). 6.5% MACE in Sema 8.0% in placebo (Risk Diff.=1.5%) SAE leading to permanent discontinuation was doubled in Sema. (16.6% in Sema, 8.2% in placebo).
Jastreboff AM, [NCT04881760], 2023, phase 2[21]	N=338, 48 weeks	Obese or overweight with weight-related comorbidities cohort without T2D Drug: Reta 1mg, 4mg, 8mg, 12mg or placebo SC injection once weekly Retatrutide=multireceptor agonist of (GLP-1+ GIP+glucagon)	Objectives: Assess efficacy of Reta on body weight loss. Primary outcome: Change in body weight (%) by week 24. Results: Wt. change at 24 weeks -7.2% (1-mg), -12.9% (4-mg), -17.3% (8-mg), & 17.5% in the 12-mg retatrutide groups, -1.6% placebo. HR peaked at 24 weeks and declined thereafter. NB: comparator should have been Tirzep, not placebo to show adding glucagon would be safe.
Aronne L, SURMOUNT-4 [NCT04660643], 2024, phase 3[27]	N=670, 88 weeks (36 weeks onward placebo could be administered)	Cohort: Obese or overweight without T2D Drug: Tirzep 10mg, 15mg Mean wt. loss 20.9%. At wk 36 randomized to continue Sema or placebo.	Objectives: Assess Tirzep effect on maintenance of body weight reduction. Primary outcome: Mean change in weight from week 36 until week 88 (%). Results: Switched to placebo group regained 14% wt. (67%)

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			continuing tirzepatide lost additional 5.5%.
Loomba R, [NCT04166773], 2024, phase 2[28]	N=190, 52 weeks	Cohort: Confirmed-MASH with liver fibrosis Drug: Tirzep 5mg, 10mg, 15mg or placebo SC injection once weekly	Objectives: Assess safety and efficacy of Tirzep as a MASH treatment. Primary outcome: Resolution of MASH without worsening of fibrosis by week 52. Results: Risk diff. 34%, 46%, & 53% at Tirzep 5-mg, 10-mg, 15-mg respectively.
Sanyal AJ, [NCT04771273], 2024, phase 2[29]	N=293, 48 weeks (24 weeks rapid-dose phase, 24 weeks maintenance phase)	Confirmed-MASH with fibrosis cohort Drug: Survo 2.4mg, 4.8mg, 6.0mg or placebo SC injection once weekly	Objectives: Assess safety, tolerability and efficacy of Survo (Dual agonist of Glucagon and GLP-1 ra)as a MASH treatment. Primary outcome: Reduction in MASH with no worsening of fibrosis by week 48. Results: risk diff. of liver fat decrease were 49%, 53%, & 43% in the 2.4mg, 4.8mg, 6.0mg Survo groups respectively. more nausea in Survo (66% vs. 23%), diarrhea (49% vs. 23%), and vomiting (41% vs. 4%); SAE were similar.
Sanyal AJ, [NCT04881760], 2024, phase 2a[30]	N=98, 48 weeks	Obese or overweight with weight-related comorbidities cohort without T2D Drug: Reta 1mg, 4mg, 8mg, 12mg or placebo SC injection once weekly	Objectives: Assess safety, tolerability and efficacy of Reta for body weight loss, assess liver fat at 24 weeks. Results: At 24 weeks, normal LF was achieved by 27%, 52%, 79%, & 86% with 1 mg, 4 mg, 8 mg, 12 mg of Reta and 0% (placebo). LF reductions were related to changes in Wt., abdominal fat and metabolic measures of insulin sensitivity.

Nota Bene: In real-world data, age at the time of GLP-1 initiation, and GLP-1 ra cessation increased the risk of major adverse cardiac events (MACE).[31] These facts strongly suggest that age and other factors can be important confounders.

The two incretins have shared functions as well as divergent functions. They share the insulinotropic function but when combined, they seem to have additive action. GIP has more powerful insulinotropic action and is responsible for 44% of the total insulin responses and GLP-1 contributes 22%. [32] We will review the role of incretins and other molecules involved in glucose homeostasis and weight management in the next section.

a. GLP-1 and GIP Mechanism of Action

When GLP-1 and GIP bind to their cognate receptors, they stimulate insulin secretion from the pancreatic β-cell through the incretin effects.[33] Both incretin-related receptors (GIPr) and GLP-1r belong to the class B family of 7-transmembrane G protein-coupled receptors (GPCR). GIP and GLP-1 share the insulinotropic actions, but other functions maybe divergent. For example, GIP stimulates

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glucagon secretion from pancreatic α-cells in hypoglycemia in healthy persons, but in T2D, the glucagonotropic function of GIP is dysregulated.[34] While GLP-1 is known to inhibit glucagon secretion possibly via somatostatin,[35] delay gastric emptying, and suppress food intake, GIP does not appear to slow gastric emptying.[36] Reports regarding GIP's role in appetite suppression are also conflicting: Animal studies and *in vitro* studies reported GIP's role in appetite suppression, [5,6] while human studies did not. [8,10] Another reason for combining GLP-1 and GIP agonists is that GIP has an anti-emetic function and counteracts nausea and vomiting evoked by GLP-1 ra.[37]

b. Amylin Mechanism of Action

Amylin, also known as islet amyloid polypeptide, is co-expressed with insulin from the pancreatic β-cells but has no insulinotropic function. For this reason, amylin is not considered an incretin. [38] Amylin reduces endogenous glucose production by suppressing glucagon secretion, slows gastric emptying, and suppresses CNS reward centers leading to weight loss. However, amylin as the name suggests, 'islet amyloid polypeptide' can self-aggregate and cause endoplasmic reticulum stress, serious cytotoxicity, and may cause β-cell death. [39] Therefore, the clinical utility of amylin is very limited.

c. Glucagon Mechanism of Action

Glucagon is a peptide with 29 amino acids and is secreted by α-cells of the pancreas. It is not an incretin but intimately participates in glucoregulation and body weight management. Glucagon receptors (GCGR) are expressed in many organs including liver, kidney and heart among other organs. The main biological function of glucagon is counter-balancing insulin in hypoglycemia. It is also involved in hepatic lipids and amino acids metabolism. Additionally, glucagon is known to enhance satiety and suppress food intake, and it has become an attractive molecule for body weight management.[40] Glucagon also promotes lipogenesis and ketone body formation from non-carbohydrate energy. In times of high energy demand, glucagon converts fatty acids to acetyl-coenzyme A via β-oxidation in the liver.[41] Additionally, glucagon activates the signaling pathway to inhibit hepatic *de novo* lipogenesis and prevent the onset of hepatic steatosis.[42] Although activation of GCGR is involved in the body weight-lowering action of oxyntomodulin (OXM), OXM's involvement in weight loss appears to be redundant with GLP-1 agonism. In T2D, however, glucagon regulation is abnormal.[43] The newly developed triple agonist, retatrutide, containes of GLP-1, GIP and GCGR agonist. However, adding 3 powerful molecules together may beget a hidden danger in a long-term usage. Let us recapitulate the benefits of incretins.

Increasing insulin secretion and improving glycemic control; Some studies also reported insulin sensitivity. However, insulin sensitivity was reported to be associated with inflammation arising from obesity. [44,45]

Suppress food intake by slowing gastric emptying and increasing satiety which lead to weight loss. [10] Interestingly, semaglutide did not delay gastric emptying assessed using paracetamol absorption in a recent trial.[46] In a murine model, GLP-1 did not slow the gastric emptying.[47] As we have stated earlier, manipulating the neurohormonal axis by incretins may be the cause of weight loss. The same pathway also reduces craving for alcohol intake. Unfortunately, when incretin's manipulation of neuronal pathways is terminated, approximately 2/3 of the lost weight was regained. [24,48]

May potentiate functional β-cell regeneration in animal or *in vitro* studies. [49] However, clear evidence of functional β-cell regeneration or proliferation in humans is lacking. [35]

May prevent bone fractures.[50] However, not all studies reported beneficial effects of incretin agonism on bone mineral density (BMD). Two randomized trials reported that exercise might be a better option for BMD than GLP-1 agonism.[51,52] Further well-conducted studies are needed.

Several reports suggest that relative hyperglucagonemia contributes to fasting and postprandial hyperglycemia in T2D, and glycemic control may be achieved by blocking glucagon action. [53] Moreover, numerous reports suggested the deleterious effects of GLP-1 ra as well as glucagon on

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heart rate and other cardiac functions. [54–57] Please refer to the "**Challenges"** section for further reading.

2. Role of Incretins in the Neurohormonal Axis of Appetite Control

Homeostatic feeding is a mechanism where energy for basic metabolic processes and survival will be obtained, while hedonic feeding is driven by sensory perception or pleasure. [58] Homeostatic feeding is tightly controlled by many molecules, hormones, and neuronal elements. These include sensing nutrients in the central nervous system (CNS), integrating afferent stimuli, reflecting the energy balance, and adjusting subsequent food intake. [59] The incretin effect is largely mediated by neuroendocrine actions and is correlated with the size of the meal. [38]

In the neuroendocrine control of food intake, the brainstem and hypothalamus are the core CNS areas because they receive, convey, and integrate peripheral signals. The area postrema (AP) and nucleus tractus solitarius (NTS) in the brainstem convey the peripheral signals, consisting of nutrients, hormones, and vagal afferent inputs, to the arcuate nucleus (ARC) of the hypothalamus. The ARC contains both orexigenic and anorexigenic neurons. The former expresses neuropeptide Y (NPY) and agouti-related peptides (AgRP) and the latter expresses pro-opiomelanocortin (POMC) and cocaine / amphetamine-related transcript (CART). They collectively process the received information and regulate eating and attain energy homeostasis. [60,61]

POMC cells activate melanocortin 4 receptor (MC4R) expressing neurons in the paraventricular nucleus of the hypothalamus (PVH) and other brain regions, thereby inhibiting food intake and increasing energy expenditure. The MC4R gene is involved in the brain's regulation of appetite and weight. Conversely, NPY/ AgRP neurons antagonize these effects. [62]

Also, leptin and serotonin are involved in regulating energy balance, appetite, and bone mass. Although both leptin and serotonin depolarize POMC neurons [63,64], there is a distinct selectivity in the responsive neurons. Namely, serotonin-responsive POMC neurons are not activated by leptin. Also, these two groups of neurons are anatomically segregated: leptin-activated POMC cells are located more laterally in the ARC than the serotonin-responsive cells. [64] Serotonin modulates the endogenous release of both agonists and antagonists of the melanocortin receptors, which are a core component of the CNS circuitry controlling body weight homeostasis. It should be noted that non-homeostatic or hedonic feeding can override this homeostatic pathway and result in overeating and obesity. Therefore, preventing hedonic feeding by food choices may be beneficial for weight homeostasis.

A sophisticated murine study reported the presence of GABAergic neurons in the dorsal vagal complex as a new player in the governing feeding behavior. [7] However, it was reported that GABAergic neurons, do not appear to express AgRP and reduce inhibitory tone to postsynaptic POMC neurons.[65] Vong's study actually challenges the recent sophisticated studies in mice and insinuates that these studies might be flawed. The authors of murine models have shown that GABAergic neurons inhibit NPY [7] or food intake. [6] Moreover, the effects of GABAergic signals are not limited to NPY/ AgRP inhibition. GABAergic signals also inhibit (POMC) neurons during fasting.[61]

Both anorexigenic neurons as well as orexigenic neurons could be inhibited, or stimulated, depending on the energy status of the host.[61] Therefore, a multitude of feeding possibilities exists even without accounting for the brain region involved. The probability of non-exclusive event involving multiple molecules with 2 possible functions (inhibited or stimulated) is impossible to estimate in animal studies. The key point is that GLP-1 and GIP receptor agonists pharmacologically manipulate the neural signals and consequently, obese individuals lose appetite as well as body weight.

Peripheral injection of fluorescently labeled liraglutide revealed the presence of the drug in the circum-ventricular organs. [66] In this study, murine brain slices showed that GLP-1 directly stimulates POMC/CART neurons and indirectly inhibits neurons expressing neuropeptide Y (NPY) and agouti-related peptide (AgRP) via GABA-dependent signaling. [66] The labeled liraglutide was

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internalized in the neurons expressing proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART).[66]

Beck also reported that the physiological activation of neuropeptides is dependent on energy availability of the host.[61,67] When energy is deprived or restricted, NPY is activated and when energy availability returns to normal, NPY synthesis returns to baseline level. Also, NPY metabolism is regulated by diet type, especially carbohydrate and fat content.[67] More recent findings support the view that the hypophagia was mediated by GLP-1 receptors in the brain.[68]

Toda summarized these facts as "arcuate melanocortin neurons consist of two distinct neuronal populations: (POMC)-expressing neurons and NPY/AgRP)-expressing neurons."[62] However, it should be noted that POMC cells are activated by energy surfeits (excess) signals and inhibited by energy deficits.[61] In other words, the energy excess or deficits are the drivers of POMC cells. So, if the person's energy state is the driver of POMC cell activation, then the energy state of humans should be controlled to activate or inhibit the POMC cells. We need to pause here to remind ourselves, that in a string of biologic actions, the earliest event is the cause. Therefore, food intake and resultant energy state of the host are the cause of POMC cell activation. Our view is supported by Beck and Rau who stated that "POMC cell stimulation or inhibition is the consequence of food intake and the resultant energy state."[61,67]

The pharmacological incretin agonism manipulates these neural axes, and control the person's appetite. However, once these neural inhibitions are lifted, weight regain can occur.[24,48] After discontinuation of tirzepatide, the study participants regained almost 2/3 of their lost weight within 4 weeks.[48] We will suggest a strategy to mitigate the weight regain after discontinuation of incretin agonists in the **FUTURE DIRECTIONS** section. These mechanisms and neurohormonal pathways are illustrated in **Figure 1.**

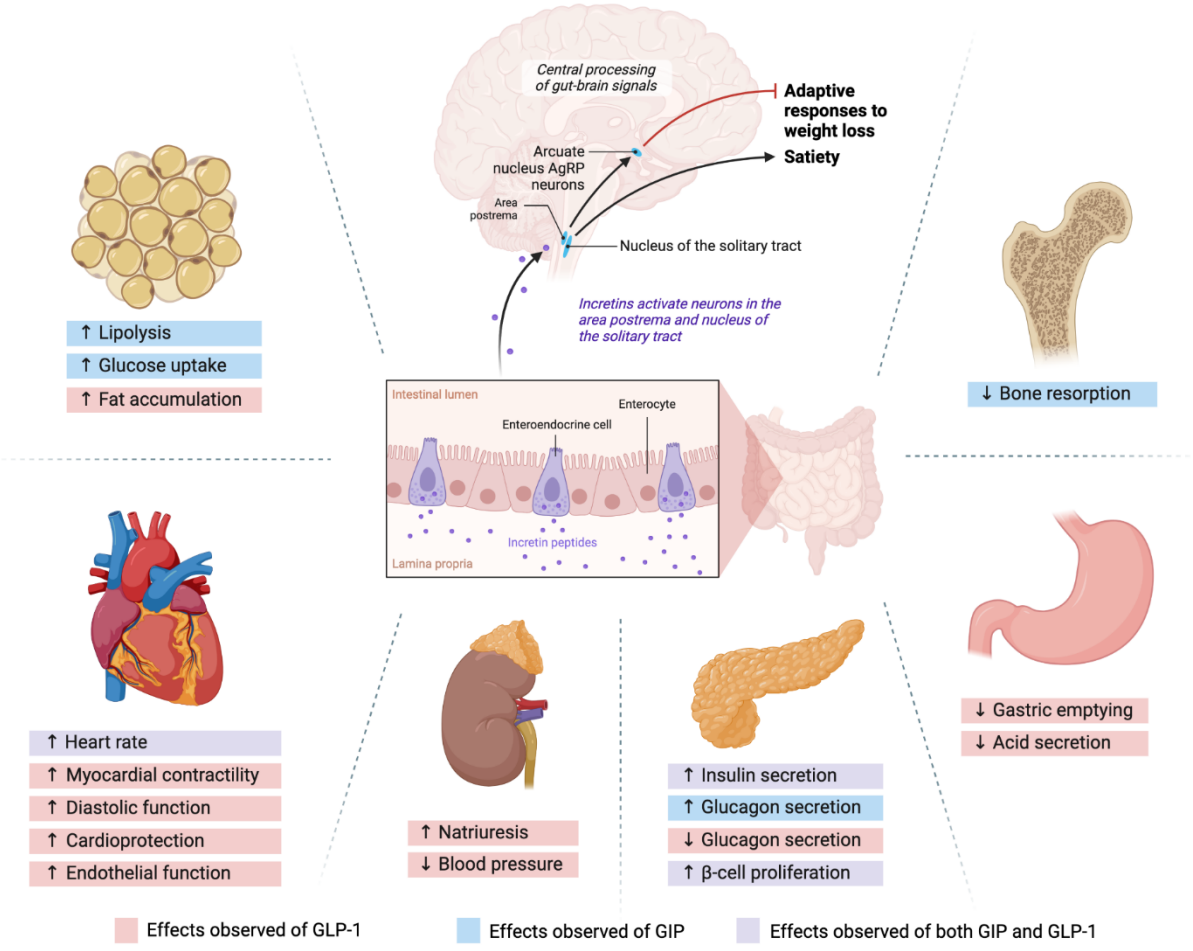

Figure 1. Neuroendocrine axis and incretin actions on multiple organs.

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3. Challenges in Incretin Agonism

a. Involvement of β-Arrestins

As stated in section1-a, incretin and glucagon receptors (GCGR) belong to the class B GPCR and their efficacy is limited by β-arrestins. [69] β-arrestin-1 (β1arrs) and β-arrestin-2 (B2arrs) are best known for their ability to mediate the desensitization and internalization of GPCRs. [70] The effect of B2arrs on insulin secretion was dominated by GLP-1R- and no clear GCGR-dependent effects are observed.[69]

B2arrs are ubiquitously expressed and function as negative regulators of GPCR signaling by inhibiting GPCR and G protein coupling, via uncoupling cyclic AMP (cAMP)/protein kinase A (PKA) signaling at physiological levels of GLP-1.[71] This is a process called desensitization which dampens insulin secretion. Next, β2-arres scaffold enzymes, phosphodiesterase and diacylglycerol kinase, degrade second messengers generated by G protein activity. This will solidify the desensitization process.[72]

At pharmacological doses, the activation of extracellular signal-related kinase (ERK)/cAMP-responsive element-binding protein (CREB) requires B2arrs. Also, GIP-dependent insulin secretion needs B2arrs in human islets.[71]

β2arres involvement is a potential drawback of incretin agonism because it will cause plateau in the drug efficacies. Avoiding B2arrs-dependent GPCR desensitization may alleviate the problem of tachyphylaxis of GLP-1 and GIP agonism.[73] The recent development of Tirzepatide is based on the previous study that revealed islets β-arrestin1 limits the insulin response of GLP-1, but not GIP, thus may not affect insulin secretion by GIP. [74]

b. β-Cell Exhaustion and Failure

Continuing stimulation of pancreatic β-cells may cause receptor downregulation and desensitization. A good example of this phenomenon is the insulin resistance in T2D patients.[75] Forcing the β-cells to secrete insulin when they are overwhelmed by insulin demand may accelerate β-cell failure as shown with sulfonylurea administration.[76] Simply put, chronic agonism eventually reduces the number of receptors leading to a condition similar to failure of function.[77] [78]

The mechanism of β-cell failure is presumed that overstimulation of β-cell increases cytoplasmic Ca^{++} levels, and persistent elevation of cytoplasmic Ca^{++} may trigger apoptosis of β-cells.[79] Under chronic agonism, some β-cells fail to differentiate properly and lose their identity.[80]

While these examples describe overstimulation of β-cells by hyperglycemia in T2D, chronic agonism by incretins may exert similar effects. [76,81,82] Indeed, animal studies reported increased pyknotic nuclei (a sign of apoptosis) of β-cells and acinar inflammation in rats given a high dose of exenatide.[83]

4. Potential Adverse Effects of Incretin Agonism

Although newer incretins appear to be much safer, increased risk of biliary disease,[12] pancreatitis,[84] bowel obstruction,[85] and gastroparesis have been noted. [86] Also an increased risk of pancreatic or thyroid cancers has been reported. [87,88] However, conflicting non-significant results on cancer risk were also observed. [89] But this study has very short follow-up (3.9 years) and compared GLP-1ra to dipeptidyl peptidase 4 inhibitors (DPP4i) which is on the causal pathway of GLP-1ra and thus, is an inappropriate comparator.

It is plausible that insulin secretion modulated by incretin agonism may affect pancreatitis and pancreatic cancer, because of their shared pathway. Indeed increased risks for multiple pathologies were reported.[90] Sodhi et al. [90] using a large health claims data set reported that the use of GLP-1ra compared with bupropion/naltrexone was associated with a 9-fold increased risk of pancreatitis, a 4.2-fold increase of bowel obstruction, and a 3.7- fold increase in gastroparesis. [90]

The cause of pancreatitis associated with GLP-1ra is hypothesized as incretin agonism may promote pancreatic hyperplasia, leading to increased pancreatic weight and exocrine duct occlusion. [91] However, the incidence of pancreatitis resulting from the incretin agonism is relatively rare and

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only large data sets such as pharmacovigilance data or insurance claims data will show significant results.

Using large historical population data, Dankner et al. reported no increase in GLP-1ra-associated pancreatic cancer incidence compared to insulin and metformin.[92] However, this study is fraught with selection bias.[92] Insulin is a well-known oncogene and this will generate selection bias. Choosing a worse comparator generate an aura of erroneously making GLP-1ras apper innocuous. [92] Further well-conducted studies are needed.

Other adverse effects such as retinopathy,[93] gastroparesis and bowel obstruction[85] were also reported. Lu and colleagues [94] explained the mechanism of intestinal obstruction as "long-term application of GLP-1ras may also elevate the release of endogenous GLP-2. GLP-2 is a cell-specific growth hormone regulating the growth of the small intestine, colonic villi and crypts, increasing villi height, and reducing antral motility.". [94] This study provides the scientific basis for the intestinal blockage and raises concerns for the long-term use of incretin agonism. [94]

Recently several cases of pancreatitis and one fatality associated with Tirzepatide [95] have been reported. As more people use this class of medications, more serious adverse events may emerge. Especially, for tirzepatide with added GIP which masks the nausea and vomiting,[37] and allows the patients to tolerate the medication better and to take it for a longer duration. This may increase more serious adverse events.

Additionally, a case of leukocytoclastic vasculitis (LCV) induced by once-weekly subcutaneous semaglutide has also been reported. [96] Although 50% of LCV can be idiopathic, immune dysregulation is presumed cause. Especially, complete resolution of the lesions shortly after the discontinuation of semaglutide proves the causal role of semaglutide on LCV. [96]

Also, GLP-1R and GCGR agonism may increase heart rate (HR) [54,57,97,98] and sympathetic tone.[99,100] Both glucagon[101] and GLP-1[102] are known to increase sympathetic tone.[103,104] Although some studies reported no increase in HR , animal studies suggest that GLP-1 acts directly on the sinus node.[55,56]

The mechanism that glucagon receptor (GCGR) activation leads to increased HR is via adenylyl cyclase activation and increases in 3',5'-c- AMP production in the myocardium. [54] For this reason, glucagon is used as an antidote for β-blocker overdose.[101] It is conceivable that glucagon is associated with sympathetic tone because hypoglycemia activates stress response and the hypothalamic-pituitary-adrenal axis. [105–108]

The mechanism that GLP-1R contributes to increased HR is reasoned that the preproglucagon neurons that mediate GLP-1-associated anorexia are situated in the brainstem to receive signals of stress. This suggests a potential link with the sympathetic nervous system. [102,103] However, another study reported that the initial increase in sympathetic tone was reversed by GLP-1R activation via by acetylcholine and nitric oxide.[109] Making it more confusing, in a human trial, the addition of liraglutide to exercise appeared to blunt the beneficial effects of exercise on left ventricular diastolic function. [52] Many systematic reviews do suggest cardiac protective effects of incretins. [110,111] However, the absolute benefit size was only a 1.5% decrease. [112] Also, a meta-analysis of the CVD benefits of dulaglutide showed that all individual studies include relative risk of 1 (no effect). This means that the results are not statistically significant. [113]

5. Future Directions

How do we harness the observed beneficial effects of incretin agonism on glucose levels and weight loss minimizing potential harm? These are the wishes of experts and patients alike. They all raised the concern for potential adverse events from a long-term use of incretin agonism, sustainability of their efficacy, and the high cost. [114–116] We concur with the experts and suggest that the remarkable weight loss and glycemic control by incretin agonism should be juxtaposed to the high cost, it's unsustainability of efficacy, and potential adverse effects. As we stated in the neuro-hormonal control of appetite section, manipulating neuronal axis will fail when the manipulation terminates. Thus, tapering GLP-1 agonism and enhancing lifestyle modification may be the key to healthy weight maintenance. It is pivotal to teach the patients so that they can make healthier food

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choices that maintain satiety [117], encouraging physical activities. A group in Denmark had done this by slowly tapering semaglutide and applying intense lifestyle modification in a trial.[118] More longer-term studies are needed emphasizing the lifestyle modifications in addition to tapering GLP-1ras.

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