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Pharmacological Advances in Incretin-Based Polyagonism: What We Know and What We Don't

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The prevalence of obesity continues to rise in both adolescents and adults, in parallel obesity is strongly associated with the increased incidence of type 2 diabetes, heart failure, certain types of cancer, and all-cause mortality. In relation to obesity, many pharmacological approaches of the past have tried and failed to combat the rising obesity epidemic, particularly due to insufficient efficacy or unacceptable side effects. However, while the history of antiobesity medication is plagued by failures and disappointments, we have witnessed over the last 10 years substantial progress, particularly in regard to biochemically optimized agonists at the receptor for glucagon-like peptide-1 (GLP-1R) and unimolecular coagonists at the receptors for GLP-1 and the glucose-dependent insulinotropic polypeptide (GIP). Although the GIP receptor:GLP-1R coagonists are being heralded as premier pharmacological tools for the treatment of obesity and diabetes, uncertainty remains as to why these drugs testify superiority over best-in-class GLP-1R monoagonists. Particularly with regard to GIP, there remains great uncertainty if and how GIP acts on systems metabolism and if the GIP system should be activated or inhibited to improve metabolic outcome in adjunct to GLP-1R agonism. In this review, we summarize recent advances in GLP-1- and GIP-based pharmacology and discuss recent findings and open questions related to how the GIP system affects systemic energy and glucose metabolism.

coagonist; diabetes; food intake; GIP; GLP-1; obesity

Identification of GIP and GLP-1 as Incretin Hormones

Starting with the observation in 1906 that intestinal mucosal extracts decrease glucosuria in diabetic patients (1), the intestine has long been recognized to control glucose metabolism (2, 3). A seminal discovery was the demonstration in 1963–1964 that the glucose-induced rise in plasma insulin is much greater when glucose is given orally relative to its infusion into the general circulation (4–6), an effect henceforth known as the incretin effect. In 1969–1970, Brown et al. (7, 8) purified a substance from a crude mucosal cholecystokinin (CCK) preparation that showed inhibitory action on gut motility and gastric acid secretion. Based on its ability to inhibit gastric acid secretion, the substance was named gastric inhibitory polypeptide (GIP) (9). In 1973, GIP was then shown to accelerate glucose-induced insulin secretion in healthy humans (10). Since the insulinotropic action of the peptide, rather than gastric mobility inhibition, prevailed at physiological doses (9–14), the peptide was renamed to glucose-dependent insulinotropic polypeptide (15). In the early 1980s, Habener et al. (16–18) then identified a glucagon-like sequence in the anglerfish preproglucagon cDNA, soon followed by the identification of two glucagon-like peptides within the preproglucagon sequence of rats (19, 20), hamsters (21), and humans (22). The novel peptides showed ~50% sequence homology to glucagon and were succinctly named glucagon-like peptide-1 and -2 (GLP-1 and GLP-2) (21). Studies by Mojsov et al. (23) and Holst et al. (24) subsequently showed that proglucagon processing results in different forms of GLP-1 in the intestine and the pancreas and that two NH_2-terminally truncated intestinal-originating forms, GLP-1

(7–37) and GLP-1(7-36NH_2), stimulate insulin secretion in the isolated perfused pancreas of pigs (24) and rats (25). Soon after the demonstration by Drucker et al. (26) that GLP-1 autonomously acts on the pancreatic β-cells to potentiate glucose-induced insulin secretion, Bloom et al. (27) then confirmed in humans that GLP-1 is a physiological incretin hormone. GLP-1 and GIP jointly account for the vast majority of the incretin effect, as verified by a largely blunted incretin effect after adjunct antagonization (28) or concomitant deletion (29) of the GLP-1 and GIP receptors (GIPRs) in mice.

Notably, while the incretin effect accounts for 50–70% of the insulin secretory response to oral glucose in healthy humans (30–34), this effect is largely blunted in individuals with type 2 diabetes (T2D) (30–33), an observation that is primarily attributed to a diminished insulinotropic action of GIP (35–41). Consequential to this, although the insulinotropic action of GIP is restored upon normalization of glycemia (42), the pharmacological interest in GIP has ever since been overshadowed by the pharmacology of GLP-1, which over the next decades emerged as a pleiotropic hormone with therapeutic value far beyond its initially described role as an insulin secretagogue. The most prominent extrapancreatic effect of GLP-1 is its ability to act on brain feeding centers to decrease body weight via inhibition of food intake, an effect first described in rats (43, 44) and subsequently confirmed in mice, birds, nonhuman primates, and humans (45). GLP-1 receptor (GLP-1R) agonists act in the brain to mediate homeostatic and hedonic feeding, with particular importance of the hypothalamic arcuate nucleus, paraventricular nucleus, hindbrain area postrema/nucleus of the solitary tract, parabrachial nucleus, and the hippocampus (46, 47). Consistent with the key role of central nervous system (CNS) GLP-1R signaling in the control of energy metabolism, GLP-1R agonists fail to affect food intake after either antagonization (48) or genetic deletion (49) of central GLP-1R in rodents. Agonism at GLP-1R in pre- and postsynaptic neurons within these aforementioned brain regions modulates the activity of the intracellular energy sensor AMP-activated kinase (AMPK), promotes enhanced neural depolarization and firing rate, and may stimulate the trafficking of alternative receptor profiles to the plasma membrane for outcome-specific optimization (50–53). These diverse mechanisms, although unknown at the time, facilitate the CNS-mediated satiety effects of exogenously administered GLP-1 in mice and obese individuals (44, 54–56).

The Use of GLP-1R Agonists for the Treatment of Obesity and Diabetes

Endogenous GLP-1 has a short circulating half-life of ∼1–2 min, which is primarily owed to proteolytic inactivation by dipeptidyl peptidase 4 (DPP4) and rapid renal elimination (57, 58). The short half-life severely limits the therapeutic potential of native GLP-1 to improve glucose metabolism, and even more so its antiobesity indication. However, in 1992, Eng et al. (59) discovered a potent GLP-1 paralog, exendin-4, in the saliva of *Heloderma suspectum* that exhibited improved circulating stability and proteolytic protection due to a unique alanine to glycine substitution at the 2nd N-terminal position of the peptide, resulting in a half-life of ∼2 h (60). The improved half-life of exendin-4 led to the pharmaceutical development of an injectable application, which demonstrated antidiabetic and certain satiety-inducing effects in patients with T2D (61–64). A variety of biochemically optimized GLP-1R agonists were subsequently developed, using alternative half-life extension strategies that include increasing the size of the peptide through either linkage of two GLP-1 molecules (dulaglutide and albiglutide), or via fatty acid acylation of the glp-1 backbone (liraglutide and semaglutide). Such fatty acid acylation allows the peptide to reversibly bind to circulating albumin, with the consequence of delayed renal excretion and the unique ability of albumin to escape endosomal degradation (65). In the case of liraglutide, C16 fatty acid acylation of the GLP-1 backbone allowed for once-daily (QD) dosing and for a greater focus on achieving antiobesogenic endpoints (66–69). Liraglutide was registered in 2014 for the treatment of obesity in adults and in 2020 for obesity in adolescents aged 12–17 years. When given over 56 weeks at the indicated dose of 3 mg QD in obese patients without diabetes, weight loss attributed to liraglutide is still in the single-digit range, but with 33% and 14% of patients losing >10% and >15% body weight, respectively (70). Semaglutide, an "advanced" form of liraglutide, differs from liraglutide in that it contains a nonnatural amino acid [aminoisobutyric acid (AIB)] at position 2 to protect from DPP4 recognition and a C18 fatty diacid to further enhance albumin binding (65, 71). These modifications result in a circulating half-life of ∼160 h and hence allow for once-weekly (QW) dosing. Semaglutide (2.4 mg) was approved by the FDA for the management of obesity in 2021 and undoubtedly has performed successfully on antidiabetic and antiobesogenic endpoints (72, 73). In phase III trials, semaglutide (2.4 mg QW treatment) over 68 weeks decreased body weight in obese patients without diabetes by −14.9%, relative to −2.4% in placebo controls (73). The continued maintenance of body weight reduction is reliant on continued treatment, as treatment discontinuation results in a rapid rebound of body weight (74). Yet impressively, the SELECT phase III trial demonstrates continued semaglutide treatment (2.4 mg) to maintain an approximate −10% reduction in body weight even over the course of up to 221 weeks, indicating the lack of tachyphylaxis and sustained weight loss during long-term treatment (75). This long-term reduction in body weight during

continued semaglutide treatment is associated with decreased occurrence of fatal cardiovascular (CV) events in nondiabetic obese patients with preexisting CV disease (75). Although Semaglutide sets the stage for a new era in antiobesity medication, its ability to decrease body weight in obese patients with concurrent diabetes is still limited, with placebo-corrected weight loss, after 68 weeks of treatment, still in the single-digit range (76). In summary, biochemically engineered long-acting GLP-1R agonists testify as a premier tool for the treatment of obesity and diabetes, but the weight loss efficacy of GLP-1R monoagonism remains limited in patients with concurrent obesity and diabetes.

Is GIPR Signaling a Valuable Pharmacological Target for the Treatment of Obesity and Diabetes?

Overshadowed by the pharmacological success of biochemically optimized GLP-1R agonists, GIPR agonism has traditionally been granted very little, to no, pharmacological value for the treatment of obesity and diabetes (45, 77). This is not only attributed to the dampened insulinotropic action of the hormone under conditions of T2D (35–41) but also to the observation that GIPR-deficient mice are protected from diet-induced obesity (78–81). In line with this observation are reports showing that GIP, particularly under low to absent levels of insulin, promotes lipogenesis and adipocyte lipid deposition via increased action/secretion of lipoprotein lipase (82–84). GIP further promotes insulin-induced glucose uptake (85–87) and conversion of glucose into lipids (85) by stimulating adipocyte GLUT4 translocation (87) and by increasing adipocyte insulin receptor affinity (85–87). In line with these data indicating that GIP has an energy-conserving nature, genetic, or pharmacological inhibition of GIPR signaling decreases intramuscular lipid accumulation in aged mice (78), and genome-wide association studies have identified common genetic loss-of-function variants in the *GIP* receptor (*GIPR*) to be associated with both higher and lower body mass indexes (88–90). Consistent with this is the observation that certain GIPR antagonists decrease body weight and food intake in diet-induced obese (DIO) mice and nonhuman primates (91), particularly when given in adjunct to GLP-1R agonism (91, 92). However, while these data argue that the GIP receptor should be inhibited rather than activated for the treatment of obesity, in the absence of insulin (or under baseline insulin levels) GIP stimulates lipolysis in isolated rat adipocytes (93) and differentiated 3T3L1 adipocytes (94) and in humans with T1D (95). This lipolytic action of GIP is mediated via its ability to promote cAMP production and can be antagonized by the addition of insulin, which inhibits cAMP production, or upon direct inhibition of adenylate cyclase (94). In line with this notion, GIPR is a $G\alpha_s$-coupled G protein-coupled receptor and hence not only stimulates cAMP production in the pancreatic β-cells (96, 97) but also in insulin-deprived isolated rat adipocytes (93) and differentiated 3T3L1 adipocytes (94). Consistent with the ability of GIP at the $G\alpha_s$-coupled GIPR to promote cAMP-driven lipolysis under low insulin conditions, adipocyte-specific DREADD-mediated $G\alpha_s$ activation similarly reduces body fat and enhances adipocyte lipolysis (98). A single administration of a long-acting fatty acid acylated (acyl) GIP further induces fatty acid oxidation in DIO mice (99), and mice with overexpression of GIP are lean and show decreased fat mass when chronically exposed to a high-fat diet (HFD) (100). In summary, GIP differentially acts on the adipose tissue by stimulating lipolysis under baseline conditions but by accelerating the antilipolytic effect of insulin under conditions of hyperinsulinemia (FIGURE 1).

Notably, data related to GIPR signaling in the adipose tissue are not undisputed, given that mice with targeted deletion of *Gipr* in the adipose tissue do not recapitulate the obesity-protecting phenotype seen in mice with global *Gipr* deficiency (101–103). Reasonable evidence further indicates that *Gipr* in the heterogenous adipose tissue is predominantly expressed in nonadipocyte cell types, including mesothelial cells and pericytes (102). The latter is implicated in the regulation of tissue vascularization (104, 105), and as such GIP promotes adipocyte lipid deposition, at least in part, by increasing adipose tissue blood flow (106, 107). However, these nonadipocyte effects of GIP on adipose tissue function cannot explain the various reports that testify to GIP's direct cell-autonomous effect on adipocyte lipid metabolism, as has been shown in 3T3L1 adipocytes (82–84, 87), as well as in adipocytes isolated from humans (83, 108) and rats (85–87, 109). Nonetheless, in contrast to mice with adipocyte-specific deletion of *Gipr* (101, 102), mice with targeted deletion of *Gipr* in the brain (99), and specifically in GABAergic neurons (110), show decreased body weight and fat mass when fed with an HFD. However, even these neuron-specific *Gipr*-deficient mice recapitulate only a mere fraction of the obesity-protecting phenotype seen in mice with global *Gipr* deficiency (78–81). However, the source of additional protection against DIO remains unknown, as tissue-specific knockdown of *Gipr* in brown adipose tissue, pancreatic β-cells, and adipose-localized immune myeloid cells all fail to recapitulate the normalization of body weight seen in the global knockout (KO) mice (FIGURE 2) (111–114). In summary, while a series of studies show GIP promotes lipid storage under conditions of hyperinsulinemia, GIPR agonism accelerates cAMP-mediated lipolysis under hypo- or normo-insulinemic conditions. Additionally, a series of genetic studies show that protection of diet-induced obesity in *Gipr*-deficient mice is partially mediated via central mechanisms but is unrelated to GIPR presence in adipose tissue.

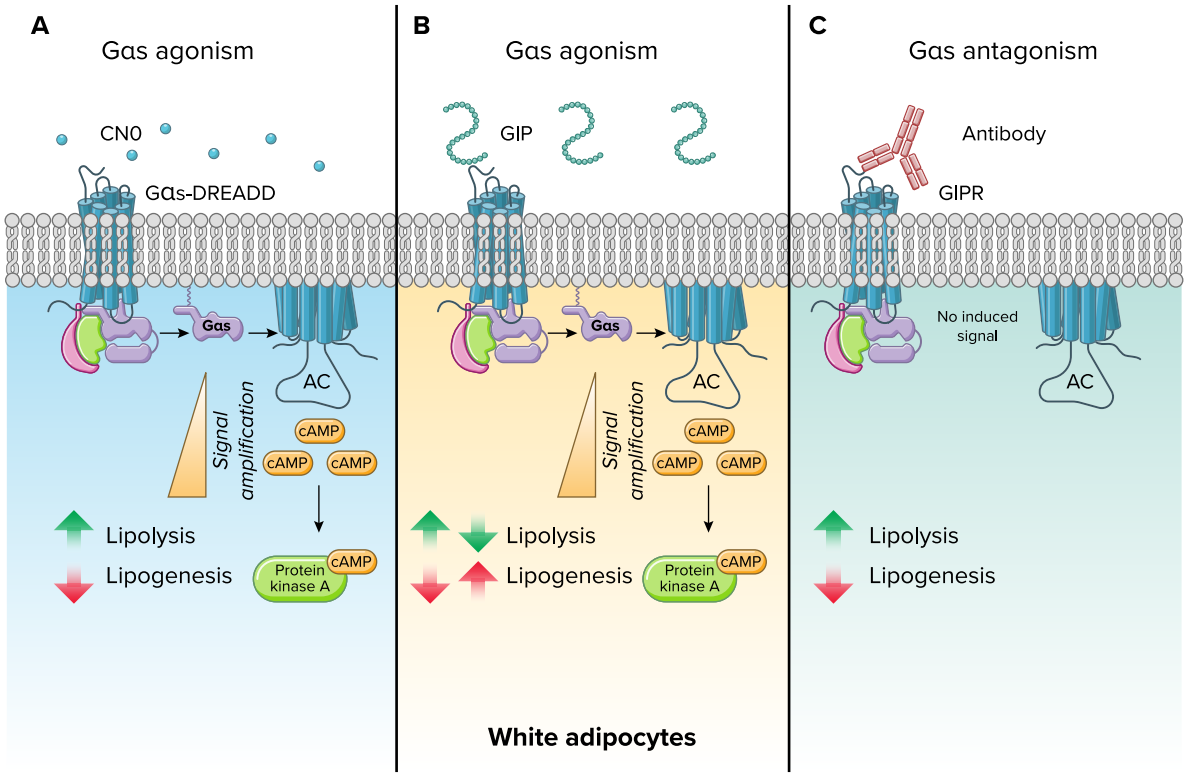

FIGURE 1. **Schematic of different activation statuses of Gα_s-coupled G protein-coupled receptors (GPCR) in white adipose tissue, and the subsequent metabolic effects**
A: agonism of the Gα_s pathway, as mediated by a white adipocyte-specific Gα_s-coupled DREADD receptor, leads to enhanced cAMP/PKA mediated lipolysis and decreased lipogenesis in DIO mice. *B*: agonism of the Gα_s pathway, as mediated by white adipocyte-specific glucose-dependent insulinotropic polypeptide receptor (GIPR), has resulted in contradictory results, with reports of enhanced lipolysis and decreased lipogenesis akin to the Gα_s-coupled DREADD receptor, and opposing reports of enhanced lipogenic and decreased lipolytic effects; additionally, there has been conflicting evidence whether GIPR agonism in the white adipocyte increases intracellular cAMP. *C*: antagonism of the GIPR, which prevents ligand binding and subsequent Gα_s pathways activation, has suprisingly been linked to increases in lipolysis and decreases in lipogenesis. AC, adenylate cyclase.

Apart from GIP effects on the adipose tissue, which seem to vary depending on the presence of insulin, long-acting GIPR agonists act in the brain to decrease body weight via inhibition of food intake (99). Chemogenetic activation of GIPR neurons in either the hypothalamus (115, 116) or the hindbrain (116) decreases food intake, and central administration of acyl-GIP into the hypothalamic third ventricle dose-dependently decreases body weight and food intake in DIO wild-type mice but not in mice with Nestin Cre-mediated neuronal loss of *Gipr* (99). In the hypothalamus and the hindbrain, *Gipr* is colocalized with vesicular GABA amino acid transporter (*Vgat*), a marker indicative of inhibitory GABAergic neurons, and deletion of *Gipr* specifically in *Vgat*-expressing neurons is sufficient to fully block the ability of acyl-GIP to decrease body weight and food intake in DIO mice (110).

It warrants clarification as to why both activation and inhibition of the GIP receptor decrease body weight and fat mass in experimental animals. A popular hypothesis is that GIPR agonism desensitizes the GIP receptor and hence leads to functional GIPR antagonism (117). Chronic GIPR agonism has indeed been shown to result in reduced GIPR sensitivity in DIO mice, and in isolated adipocytes (103), but similar results have been shown for GLP-1 in rat insulinoma INS-1 cells (118) and for both incretins in hamster β-cell HIT-T15 cells (119). In addition, even chronic treatment of DIO mice with acyl-GIP does not decrease the expression of *Gipr* in the hypothalamus or the adipose tissue (99). While there is currently no evidence indicating that GIPR agonism decreases body weight through functional GIPR antagonism, it seems possible that GIPR agonists and antagonists affect body weight through different mechanisms and target tissues, with long-acting GIPR agonists acting on brain satiety centers to affect food intake, while GIPR antagonists may compete with endogenous GIP in the periphery to inhibit the lipogenic action of endogenous GIP in the adipose tissue (120). Another possibility is that while GIPR agonism acts centrally to decrease food intake via activation of GABAergic neurons (110), GIPR antagonism may inhibit GABAergic input into the anorectic GLP-1R positive glutamatergic neurons, thereby reducing food intake via unrestrained glutamatergic effect analogous to GLP-1R agonism. Consistent with this is the observation that GIPR antagonists primarily decrease food intake when given together with GLP-1 (91, 92), while GIPR agonists also decrease food intake and body weight in mice deficient for GLP-1R (99, 121). While these hypotheses warrant experimental verification,

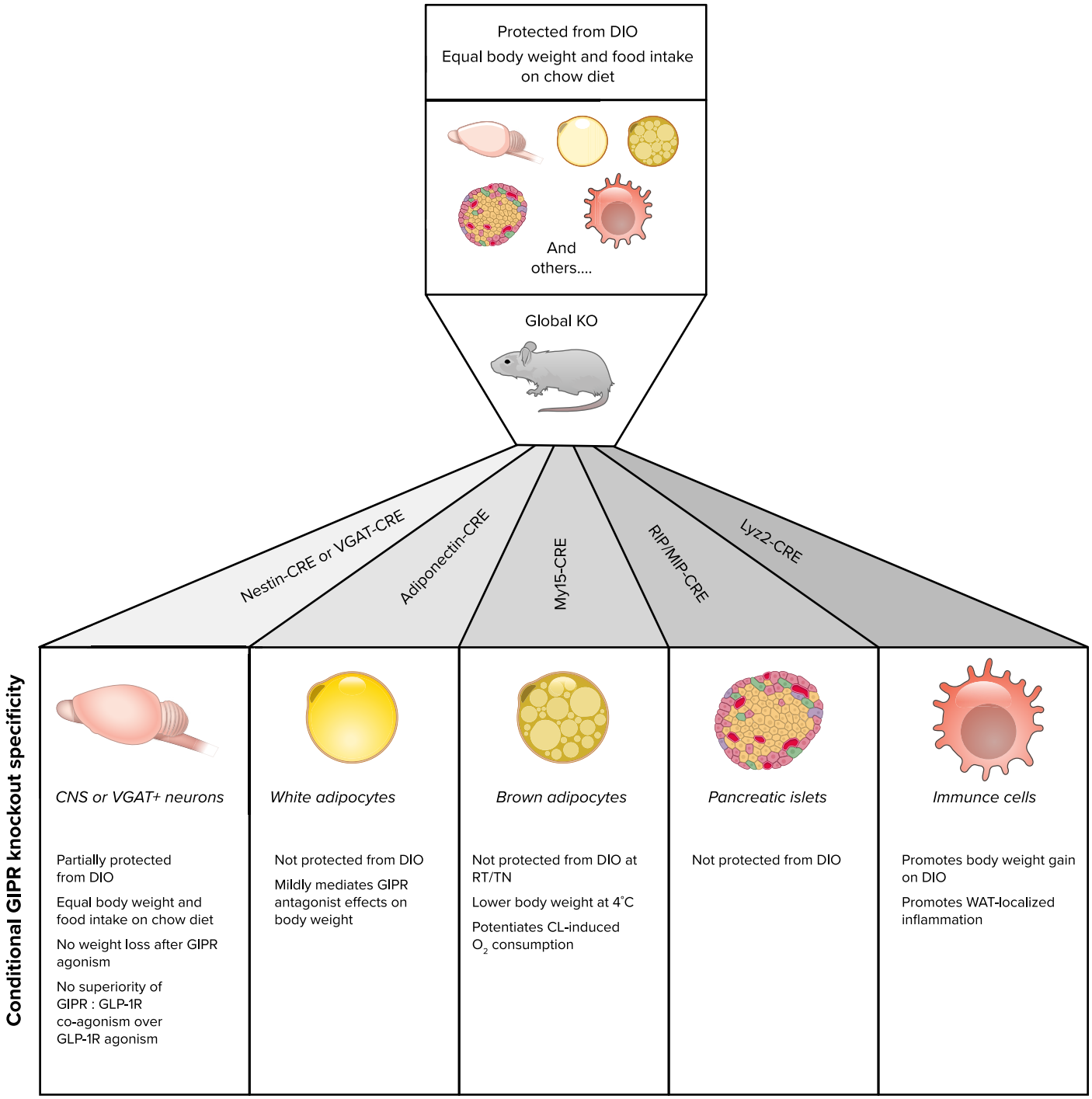

FIGURE 2. **Schematic on the metabolic phenotype of conditional and global glucose-dependent insulinotropic polypeptide receptor (Gipr) knockout (KO) mice**
Simplified chart describing the role of tissue-specific GIPR knockouts on the degree of protection against body weight gain under chow and high-fat diet or its requirement for mediating GIPR-based pharmacological-induced weight loss. CNS, central nervous system; DIO, diet-induced obese; GLP-1R, glucagon-like peptide-1 receptor; RT/TN, room temperature/thermoneutral; VGAT, vesicular GABA amino acid transporter; WAT, white adipose tissue.

GIPR agonism clearly depends on GIPR signaling in the CNS to decrease body weight and food intake (99, 110), and while the greatest body weight-lowering effects of GIPR antagonists are observed using antibody-based GIPR antagonists (91), antibodies have generally a very limited ability to reach the brain (122). Nonetheless, when administered directly into the brain, antibody-based GIPR antagonists also decrease body weight in DIO mice, an effect paralleled by improved leptin sensitivity (123). The obesity-protecting phenotype seen in mice with global GIPR deficiency however cannot be solely explained by improved leptin sensitivity, as deletion of *Gipr* in leptin-deficient *ob/ob* mice reduces body weight relative to *ob/ob* controls (81).

Another open question is why pharmacological GIPR agonism has not been as clearly delineated

relative to GLP-1R agonism. An important realization is that the GIP system is evolutionary less conserved than the GLP-1 system, and human GIP is only a weak and partial agonist at the mouse GIP receptor (124). The importance of this has recently been demonstrated for the GIPR:GLP-1R coagonist tirzepatide, which is based on the human GIP sequence and stimulates islet insulin secretion in mice predominantly via the GLP-1 receptor but in human islets via the GIP receptor (125). In light of this notion, and given that neither human GIP nor tirzepatide is capable of fully activating the mouse GIP receptor (124), it is hence not overtly surprising that tirzepatide decreases body weight in mice exclusively via the GLP-1 receptor (126). Consequentially, as tirzepatide shows only weak agonism at the mouse GIP receptor, these data do not allow for the conclusion that tirzepatide also decreases body weight in humans exclusively via the GLP-1 receptor. Consistent with this, the GIPR:GLP-1R coagonist MAR709 is far more potent at the mouse GIP receptor relative to tirzepatide, and treatment of DIO mice with MAR709 leads to greater weight loss and further inhibition of food intake relative to mice treated with a pharmacokinetically matched GLP-1R agonist (99, 110). In line with intact GIPR signaling in MAR709, its superiority to a matched GLP-1R agonist vanishes in mice with neuronal loss of *Gipr* (99) or when *Gipr* is specifically deleted in *Vgat*-expressing GABAergic neurons (110). In summary, GIPR agonists that are based on the human GIP sequence (including tirzepatide) are insufficiently potent at the mouse GIP receptor and are hence incapable of studying the mode-of-action of GIP-based drugs in rodents. Nonetheless, if sufficiently active at the mouse receptor, GIPR agonism decreases body weight via inhibition of food intake (99, 110) and can be a vital constituent in unimolecular formulations with GLP-1R agonism (99, 110, 127, 128). Consistent with this, central coadministration of low-dose GIP and GLP-1 synergistically reduces food intake (127), and this effect correlates with synergistic increases in neural activation of pro-opiomelanocortin in the hypothalamic arcuate nucleus (129).

Fatty acid acylation of GLP-1 and GIP has further proven to be an appreciable biochemical tool to enhance CNS-driven satiety effects of both GLP-1 and GIP. Whether such fatty acid acylation increases metabolic outcome simply by the extended half-life, or maybe also due to increased brain penetrance, and exposure of deeper brain structures implicated in energy metabolism control, remains to be determined (130, 131). The observation that neither liraglutide (132) nor semaglutide (133) can cross the blood-brain barrier, however, suggests that these molecules rather act on the circumventricular organs to inhibit food intake, and recent evidence indicates that this is also the case for GIP (110, 134). Nonetheless, the body weight-lowering effects of acyl-GIP are preserved in GLP-1R KO mice (99, 121) but vanish in mice with deletion of *Gipr* in either CNS neurons (99) or specifically in GABAergic neurons (110), hence clearly demonstrating the involvement of central GIPR signaling in mediating these effects. In summary, there is ample evidence indicating that GIP as a single or combination therapy decreases food intake via central mechanisms, that these effects are GLP-1R independent, and that these effects are likely enhanced via acylation.

The pharmacological value of GIP is not restricted to its insulinotropic and body weight-lowering effects. GLP-1R monoagonism is frequently associated with gastrointestinal (GI) adverse effects, predominantly nausea and vomiting (135, 136). Adverse GI effects are predominantly observed in the early treatment period and can be minimized by careful gradual dose escalation (137, 138). The increased rate of GI adverse events, despite plateauing metabolic benefits, caps the maximal tolerable dose of semaglutide at 2.4 mg (73). This registered dose of semaglutide is chosen based on projected pharmacokinetic modeling of a daily 0.4-mg dose, which exhibited greater than double the GI events leading to trial discontinuation than the next lower dosage of 0.3 mg/day (139). It appears that GIPR agonism in adjunct to GLP-1R agonism mitigates the GI adverse event profile of GLP-1R pharmacology to ultimately increase both patient compliance and maximal practical dosage. Coadministration of both GLP-1 and GIP eliminates the occurrence of GLP-1-induced emetic episodes in musk shrews (140), demonstrating GIP's capacity for complimentary antinausea effects. In line with the antiemetic effect of GIP, the maximal dosing of QW tirzepatide is as high as 15 mg, with an adverse event and discontinuation profile comparable to QW semaglutide at 2.4 mg (76, 141). However, given that tirzepatide is a 5:1 unbalanced GIPR:GLP-1R coagonist that favors the GIP receptor, it warrants clarification how much of the greater tolerability originates from the demonstrated lower potency of tirzepatide at the GLP-1 receptor (142–144) and/or from the antiemetic effect of GIPR agonism (140). Also, the mechanism behind the antiemetic effects of GIPR agonism warrants further clarification. Recent evidence indicates that the anorectic and aversive effects of GLP-1R agonism are mediated via hindbrain CCK neurons, and although GIP does not directly target these neurons, GIPR agonism reduces conditioned taste avoidance through diminished GLP-1-induced activation of these neurons (134). The area postrema/nucleus of the solitary tract is hence suggested to mediate satiety and satiety-related emesis, and single-cell transcriptomic profiling of the dorsal vagal complex has revealed that a significant portion of GABAergic neurons in this region express *Gipr* (145, 146). It may be coincidental that also the body weight-lowering effect of GIPR agonism is mediated by *Gipr* expressing GABAergic neurons (110), and these neurons may hence not only contribute to the body weight-lowering effect of the GIPR:GLP-1R coagonist

tirzepatide but also explain its greater tolerability, and hence the allowance of higher doses, relative to semaglutide. In summary, central GIPR agonism not only decreases body weight via centrally mediated inhibition of food intake, it further decreases the emetic effect of GLP-1R agonism, an appreciable observation that may accelerate tolerability and higher dosing of GLP-1R agonism.

Performance of Tirzepatide versus Semaglutide on Body Weight in Humans

Ironically, while no GIPR antagonist has yet been approved for the treatment of obesity, GIPR agonism has proven remarkably efficacious in accelerating the metabolic outcome of GLP-1R agonism, either as cotherapy (127) or in unimolecular formulation (127, 128). The first unimolecular GIPR:GLP-1R coagonist was developed in 2013 by DiMarchi et al. (127). The 1st generation of these molecules (NNC0090-2746/MAR709) outperformed exendin-4 and liraglutide in body weight reduction when given at equimolar doses in obese rodents (127). The GIPR:GLP-1R coagonist tirzepatide has been derived from the GIP backbone and favors GIPR over GLP-1R by approximately fivefold in binding affinity, and ~13-fold in a cAMP reporter assay (128). In vitro models have demonstrated tirzepatide to activate both the human GLP-1R and GIPR differentially within subsequent steps of the signal cascade to minimally recruit β-arrestin to the GLP-1R, to possess differential GLP-1R internalization and endosomal trafficking dynamics, and to ultimately potently reduce body weight in DIO mouse models (FIGURE 3) (128, 142, 143). The beneficial metabolic effects of tirzepatide have been verified in a series of human studies (147–150) and have resulted in the approval of tirzepatide for the treatment of T2D in 2022 and for the management of obesity in 2023. Very recently, the metabolic efficacies of tirzepatide and semaglutide were compared in real-world settings in patients with obesity (151). The study included data from over 41,000 individuals, from which 32,030 received semaglutide and 9,193 tirzepatide. After 1 year of treatment, weight reduction ≥5% was achieved in 81.8% and 64.6% of patients receiving either tirzepatide or semaglutide, while weight loss ≥10% was achieved in 62.1% and 38.0%, and ≥15% in 42.3% and 19.3% of patients (151). The superiority of tirzepatide over semaglutide was reflected by additional weight loss of −2.3% after 3 months of treatment, −4.3% after 6 months, and −7.2% after 12 months, with no changes between tirzepatide and semaglutide on the occurrence of GI adverse effects at any time point (151). Collectively, these data demonstrate that tirzepatide outperforms semaglutide in body weight endpoints without compromising its tolerability. In line with these data, the SURMOUNT-1 trial demonstrates −11.9%, −16.4%, and −17.8% placebo-corrected body weight loss at the used doses of 5, 10, and 15 mg of tirzepatide in nondiabetic obese patients over the course of 72 weeks of treatment (152). The lowest dose (5 mg) hence achieves roughly the same degree of body weight reduction as semaglutide 2.4 mg (−12.4%) (152). Interestingly, when considering the 10-mg and 15-mg groups pooled, the averaged placebo-corrected body weight loss for tirzepatide is −17.1% with 6.6% patient discontinuation due to adverse events out of a total 1,266 total patients. This is compared to semaglutide, in which out of 1,306 total patients, a placebo-corrected −12.4% reduction in body weight was achieved with 7.0% patient discontinuation (73, 152). Together, this suggests an approximate 4.5-5% enhancement in body weight loss with 10/15 mg tirzepatide relative to 2.4 mg semaglutide, with approximately the same degree of adverse events that lead to treatment discontinuation. These findings suggest that the chemical structure of tirzepatide, whether it be the GIP-dependent engagement of complementary satiety signals, GIP's potential for antiemesis-mediated escalation of GLP-1 component dosage, or intrinsic GLP-1R biased agonism, or all of them, leads to substantial improvements in body weight-lowering efficacy in nondiabetic obese individuals. Similarly, the SELECT phase III clinical trial with semaglutide demonstrates an approximate −10% reduction in body weight maintained over a maximum of 221 weeks, and the SUPRASS 4 phase III trial reports tirzepatide's maintenance of body weight loss at approximately −26% through 88 weeks (75, 153). Importantly, in obese patients with concurrent T2D, 72 weeks of 10-mg and 15-mg tirzepatide treatment resulted in −9.6% and −11.6% reduction in body weight, which is almost double that of the −6.2% by semaglutide in 68 weeks (76, 141). Similarly, in the SURPASS trials which evaluated the antidiabetic capacity of tirzepatide in patients with T2D, secondary end point changes from baseline body weight by 15 mg tirzepatide were as follows: −11.1% (SURPASS 1: 40 weeks), −13.1% (SURPASS 2: 40 weeks), −13.7% (SURPASS 3: 52 weeks), −12.8% (SURPASS 4: 52 weeks), and −9.2% (SURPASS 5: 40 weeks). Together, the SURPASS 1–5 and SURMOUNT 2 clinical trials suggest a double-digit average of induced body weight loss in T2D individuals, despite slight differences in trial durations (147–150, 154). As mentioned, it is unknown why tirzepatide performs better on weight loss within the context of T2D; however, despite the aspects of GIP's antiemetic allowance of GLP-1 component dose escalation, and the potential for intrinsic GLP-1R biased agonism, it is considerable that the plateau in maximum HbA1c reduction by tirzepatide occurs within 24 weeks, while reductions in body weight do not plateau until week 60 (141). Hence, according to the potential for GIPR resensitization during recovery toward normoglycemic conditions, these additional weight loss benefits as time continues may be attributed to the synergistic action

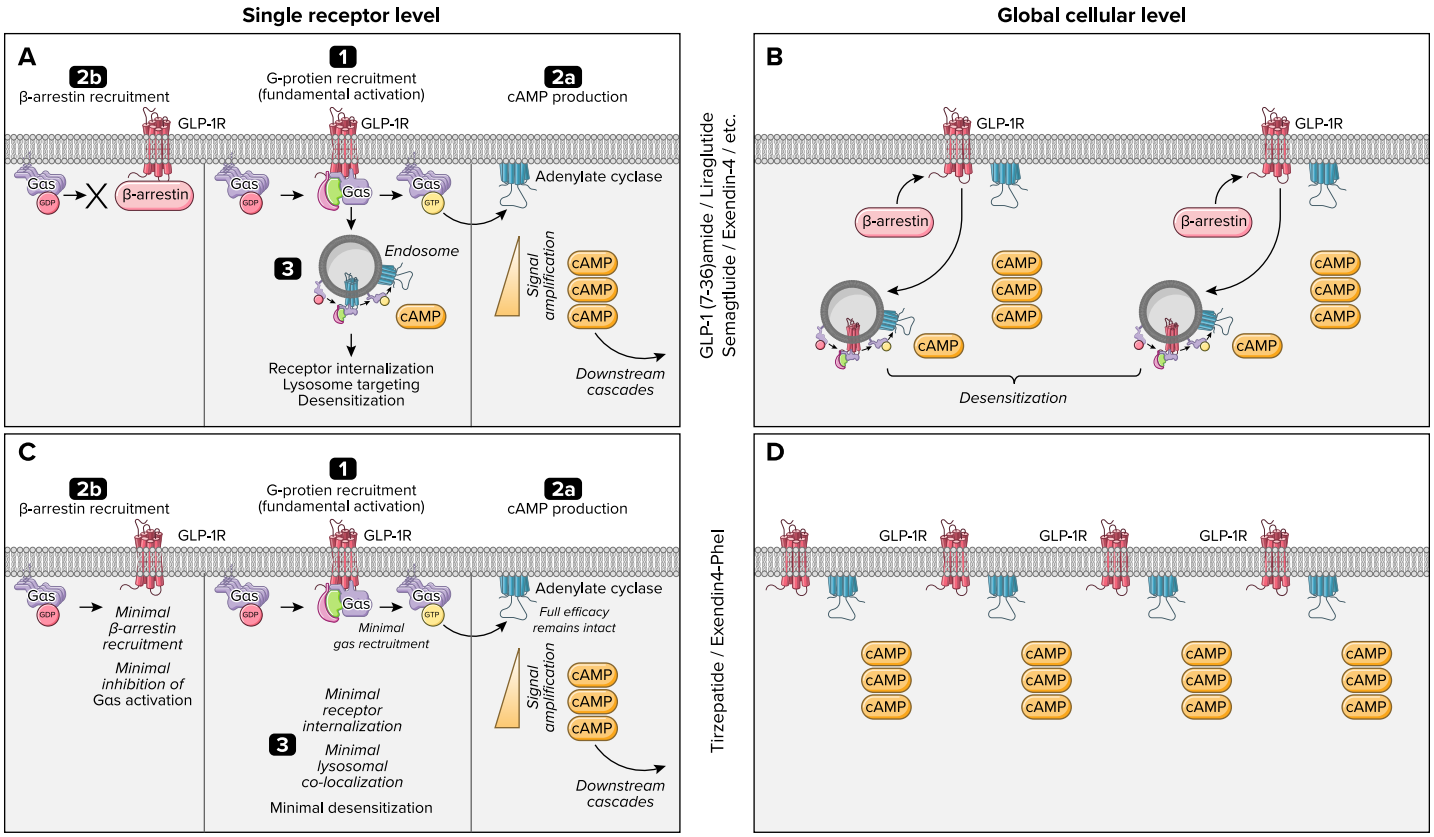

FIGURE 3. **Tirzepatide as a "super" glucagon-like peptide-1 receptor (GLP-1R) agonist?**
A–D: simplified schematic describing the alternative intracellular signaling and trafficking dynamics of the GLP-1R at the single receptor level or the global cellular level elicited by GLP-1R monoagonists such as GLP-1-(7-36NH_2) and semaglutide (*A* and *B*) or tirzepatide and exendin4-Phe1 (EX4-Phe1) (*C* and *D*). GLP-1R signaling and trafficking dynamics following ligand binding and receptor activation by mono-agonists such as GLP-1-(7-36)amide and semaglutide. *A1*: GLP-1R activation on the level of direct G-protein interaction is maximal, which includes maximal G protein-coupled receptor (GPCR)-mediated GDP to GTP exchange on the $G\alpha_s$ subunit, and maximal recruitment of GDP-bound $G\alpha_s$ to the GLP-1R for continued signaling. *A2a*: the "activated" GTP-bound $G\alpha_s$ is subsequently recruited to adenylate cyclase where it stimulates the amplified production of cAMP. *A2b*: Simultaneously, β-arrestin is recruited to the GLP-1R to facilitate a braking mechanism on continued GDP-bound $G\alpha_s$ recruitment to the ligand-bound GPCR to prevent further signaling. *A3*: internalization carries the GLP-1R away from the plasma membrane into the intracellular space, where it continues to transiently signal but also is redirected into desensitizing endolysosomal pathways. *B*: global schematic view at the cellular level of total GLP-1R dynamics resulting from the sum of unique single receptor signaling and trafficking dynamics elicited by GLP-1R agonists such as GLP-1-(7-36)amide and semaglutide. *C*: GLP-1R signaling and trafficking dynamics following ligand binding and receptor activation by "biased" GLP-1R agonists such as tirzepatide and EX4-Phe1. *C1*: GLP-1R activation on the level of direct G-protein interaction is minimal. *C2a-C2c*: maximal cAMP signaling efficacy (*C2a*) is achieved through minimal β-arrestin recruitment brake on signaling (*C2b*) and greater GLP-1R retention at the plasma membrane leading to less endolysosomal colocalization and higher receptor exposure to further extracellular ligands (*C2c*). *D*: global schematic view at the cellular level of total GLP-1R dynamics resulting from the sum of unique "biased" single receptor signaling and trafficking dynamics elicited by tirzepatide and exendin4-Phe1.

between GLP-1R and GIPR coagonism on reductions in food intake and body weight (42, 127–129). In summary, tirzepatide outperforms semaglutide to achieve greater weight loss, but it warrants clarification in human studies if and to what extend GIPR agonism contributes to better weight loss efficacy and enhanced tolerability.

GLP-1R Agonism/GIPR Antagonism

Notably, genetic or pharmacological modulation of the GIP system, whether through loss/gain-of-function or agonism/antagonism, results in different degrees of weight loss and protection from obesity (100, 155). In particular, antimurine GIPR antibodies (muGIPR-Ab) and peptide-based GIPR antagonists, while not strong stimulators of body weight loss in DIO mice, hint at some degree of induced weight loss or protection against the onset of obesity (91, 92). Nonetheless, it is clear that antibody- or peptide-based GIPR antagonist coadministration with GLP-1R monoagonists synergistically reduces body weight (91, 92). In DIO mice, liraglutide coadministration with a muGIPR-Ab (BWΔ: −27%) was superior to liraglutide alone at 80 nmol/kg (−15%) (91) and semaglutide coadministered with a peptide-based GIPR antagonist (−28%) outperformed semaglutide alone at 3 nmol/kg (−12%) (92). As an antihuman GIPR antibody (hGIPR-Ab) was found to reduce body weight both as a single therapy and as a combinatorial therapy with dulaglutide in nonhuman primates (91), a DPP4-protected GLP-1 was conjugated with a GIPR antibody (GIPR-Ab/GLP-1) and developed into the molecule AMG 133, exhibiting a half-life between 5 and 9 days depending on the species and route of administration (156). In DIO mice, mGIPR-Ab/GLP-1 reduced body weight by approximately −30%, approximately double that of GLP-1 conjugated to a nonspecific antibody (156). Similar findings were obtained in obese nonhuman primates, in which 2.5 mg/kg of hGIPR-Ab/GLP-1 induced

approximately −10% body weight loss after 43 days of treatment (156). Interestingly, at the GLP-1R, both the conjugated muGIPR-Ab/GLP-1 and hGIPR-Ab/GLP-1 are prominently less potent than unconjugated GLP-1 for cAMP production, a profile similar to that of tirzepatide (128, 156). However, when both GLP-1R and GIPR are coexpressed, the cAMP potency of the antibody-GLP-1 conjugate is surprisingly greater than that of the unconjugated GLP-1, in which the improvement is suggested to occur via enhanced ligand proximity via GLP-1R/GIPR colocalization and altered endosomal signaling (156). Indeed, it is suggested that GLP-1R and GIPR interactions at the plasma membrane upon GLP-1R agonism allow for a unique signaling profile, which is demonstrated as a reduction in both $G\alpha_q$ and β-arrestin recruitment to the GLP-1R, while $G\alpha_s$ activity remains fully intact (157). Although clinical data has not yet been published, a press release from Amgen has indicated AMG 133 to achieve up to −14.5% body weight reduction after 12 weeks in a phase I clinical trial and has additionally indicated the start of a phase II clinical trial in early 2023 (158). However, questions remain as to how antagonism of the GIPR will influence the adverse event profile of GLP-1R agonism, particularly considering the potential for GIPR agonism's involvement in antiemetic effect. In summary, GIPR agonism and antagonism both have therapeutic potential in adjunct to GLP-1R agonism, but it warrants clarification whether GIPR antagonism promotes its additional weight loss efficacy via GLP-1R-dependent or -independent mechanisms.

Is Tirzepatide a "Super" GLP-1?

A consideration of the enhanced efficacy of tirzepatide over semaglutide is that tirzepatide, for all intents and purposes, may be an incidental super GLP-1R agonist due to a signaling bias incurred by amino acid modifications implemented into the peptide backbone originally meant to confer dual agonism. Tirzepatide does activate the human GIPR, but this attribute is suggested to be inconsequential to the in vivo metabolic benefits relative to its biased attributes at the GLP-1R (142–144). Interestingly, at the GLP-1R, tirzepatide recruits minimal β-arrestin despite a concurrent cAMP E_{max} equivalent to that of semaglutide and GLP-1 (142, 143). Additionally, minimal GLP-1R internalization from the plasma membrane into the intracellular space, and the subsequent reduction in lysosomal targeting, suggest tirzepatide's influence on GLP-1R trafficking to be protective against canonical G protein-coupled receptor (GPCR) desensitization (142, 143). Together these attributes indicate a capacity for tirzepatide to maximally stimulate a cAMP response, recruit minimal signaling inhibition, and simultaneously retain more GLP-1R at the plasma membrane (FIGURE 3). In relation, a Phe1-substituted exendin-4 (EX4-Phe1) peptide demonstrates super efficacy for both antiglycemic and antiobesogenic measures (159–161). Similar to tirzepatide at the GLP-1R, EX4-Phe1 exhibits maximal cAMP efficacy and minimal β-arrestin recruitment, receptor internalization, and receptor desensitization (159, 160). EX4-Phe1-induced insulin secretion within the rat β-cell model INS-1 832/3 outperforms exendin-4, liraglutide, dulaglutide, and semaglutide, suggesting a superior capacity to elicit an insulinotropic response (159). In vivo administration of the unacylated EX4-Phe1 evidences superior glucoregulatory control relative to exendin-4 during glucose tolerance tests (GTT) performed 4 hours and 8 hours after peptide administration (159). Similarly, in a GTT performed 72 hours posttreatment, an acylated version of Ex4-Phe1 outperforms a PK-matched alternative analog of exendin-4 characterized as favoring β-arrestin recruitment and GLP-1R internalization, suggesting enhanced long-term glucoregulatory efficacy of EX4-Phe1 to be likely due to sustained receptor sensitization and a lack of receptor internalization (160). Importantly, dose escalation of 10 to 20 nmol/kg EX4-Phe1 over the course of 15 days demonstrates superior body weight-lowering effects relative to the aforementioned exendin-4 analog that favors β-arrestin recruitment and GLP-1R internalization, henceforth suggesting minimal β-arrestin recruitment and GLP-1R internalization as a successful strategy for enhancing GLP-1R-centric body weight-lowering effects (160). This strategy has further been refined as the GLP-1R monoagonist SRB107 and has hinted at promising pre-clinical results (162). Importantly, with respect to tirzepatide's enhanced efficacy relative to semaglutide, EX4-Phe1 cotreatment with a DPP4-protected GIP analog in DIO mice demonstrates superior enhancements in body weight reduction relative to EX4-Phe1 alone, suggesting GIPR coagonism to remain a potentiator of metabolic improvements even within the context of super GLP-1R agonism (163). In summary, it warrants clarification if and to what extent the favorable GLP-1R trafficking and β-arrestin recruitment profile of tirzepatide contributes to its observed greater weight loss efficacy, and how GIPR co-agonism likely contributes to a vital biochemical liaison with biased GLP-1R agonism.

Chronic GIPR Agonism as a Driver of Functional Antagonism?

A hypothesis to explain the enhanced efficacy attributed to the GIPR:GLP-1R coagonist tirzepatide is the suggestion that the onset of chronic GIPR agonism desensitizes the GIPR as to no longer elicit intracellular responsiveness. Through this functional antagonistic effect, tirzepatide's long-term mechanism is suggested to be analogous to coadministration of a GLP-1R monoagonist and a GIPR antagonist. GPCR desensitization over a longer course of action (ie. repeated drug exposure) is generally mediated by receptor internalization, ubiquitination, lysosomal targeting, and ultimately receptor degradation (164). Interestingly, there has been disagreement as to the extent of tirzepatide-induced

GIPR internalization (142, 143). Nonetheless, native GIP (1–42) demonstrates an ability to induce GIPR receptor internalization, while related variants including a Gly2-subtituted GIP and an AIB2-subtituted "acyl GIP," do not (142, 165). Relative to the GLP-1R however, the ligand-bound GIPR internalizes less, internalizes slower, is completely absent from Rab5+ early endosomes, exhibits reduced endosomal signaling and enhanced receptor recycling, and is minimally targeted to lysosomes for degradation (166). In short, it seems the GIPR is capable of undergoing some ligand-induced internalization; however, this effect does not result in substantial lysosomal targeting, and the degree of internalization seems to be especially sensitive to ligand modifications. These reflexive properties of GIPR trafficking bring into question canonical GPCR degradation as a mediating pathway to the occurrence of GIPR functional antagonism. Nonetheless, it is important to not discount differential GIPR dynamics inherent within alternative tissue types and to consider the role GIPR variants may have on these trafficking processes (167, 168).

Alternatively, GIPR desensitization has been suggested to occur via persistent β-cell membrane depolarization, an attribute representative of β-cells in the diabetic state (169). In this model, persistent membrane depolarization desensitizes the $G\alpha_s$-cAMP pathway, while alternatively preserving the efficacy of the $G\alpha_q$-IP_3 pathway (169). Indeed, the GLP-1R is both $G\alpha_s$ and $G\alpha_q$ coupled while the GIPR is only $G\alpha_s$-coupled, giving potential reason to GIPR's observed inefficacy, and a potential Gαq-mediated explanation for GLP-1R's continuing glucoregulatory efficacy, in the human diabetic state. Regarding an alternative pathway toward agonism-induced GIPR functional antagonism, it is speculatively possible that chronic concurrent agonism of both the GLP-1R and GIPR $G\alpha_s$ subunits in β-cells desensitizes $G\alpha_s$ action and thus further GIPR agonism. Yet, this analogous situation of agonism-induced functional antagonism due to $G\alpha_s$ desensitization has yet to be fully explored.

Conclusions and Future Directions

With the discovery, clinical implementation, and/or commercialization of semaglutide, tirzepatide, and AMG 133, the ground has been broken in implementing the next era of antidiabetic and antiobesogenic pharmacology. Despite major clinical success, further refinement in both understanding and approach will be needed to open pathways for future investigation, not only toward higher understanding of the associated biological dynamics, but also in the hope of optimizing toward more advanced next-generation therapeutics. Advancements in GLP-1R biology have yielded fruitful therapeutic value that has only now escalated toward revolutionizing the treatment of diabetes, obesity, and its comorbidities. Along the way, proteolytic protection and half-life extension have been key to capturing the antiobesogenic efficacy of the GLP-1 peptide. In the way these modifications are critical to the success of semaglutide, we are similarly now acknowledging the profound potential of polypharmacology in maximizing the therapeutic utility of GLP-1-centric approaches. While semaglutide has set the stage for clinical GLP-1R monoagonist approaches against diabetes and obesity, tirzepatide and AMG 133 represent novel options that pertain to GIPR-mediated complimentary enhancements of GLP-1R efficacy. Dual or triple modes of action in therapy, while allowing maximal benefit, exponentially increase the difficulty in understanding the mechanisms underpinning their enhanced efficacy. For tirzepatide, while the mechanistic resolution of GIPR biology is coming of age, the pharmacological utility of GIPR is still under debate and leaves to question, what mediates tirzepatide's superior effectiveness: synergies of GLP-1R/GIPR coagonism, synergies of GLP-1R/GIPR functional antagonism, or biased GLP-1R agonism? Similarly to AMG 133, how will antagonism of a canonical $G\alpha_s$-coupled system involved in multiple systemic processes that include satiety, emesis, and adipocyte lipid metabolism counterintuitively amplify a therapeutic GLP-1 receptor response? There are many advancements and questions listed and not listed here that will gratuitously fill research advancement for the coming years. ■

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A.N. prepared figures; A.N. and T.D.M. drafted manuscript; A.N. and T.D.M. edited and revised manuscript; A.N. and T.D.M. approved final version of manuscript.

References

1. Moore B. On the treatment of diabetus mellitus by acid extract of duodenal mucous membrane. *Biochem J* 1: 28–38, 1906. doi:10.1042/bj0010028.

2. Zunz E, La Barre J. Contributiona a l'etude des variations physiologiques de la secretion interne du pancreas: relations entre les secretions externe et interne du pancreas. *Arch Int Physiol Biochim* 31: 20–44, 1929.

3. La Barre J. Sur les possibilités d'un traitement du diabète par l'incrétine. *Bull Acad R Med Belg* 12: 620–634, 1932.

4. Arnould Y, Bellens R, Franckson JR, Conard V. Insulin response and glucose-C14 disappearance rate during the glucose tolerance test in the unanesthetized dog. *Metabolism* 12: 1122–1131, 1963.

5. Elrick H, Stimmler L, Hlad CJ Jr, Arai Y. Plasma insulin response to oral and intravenous glucose administration. *J Clin Endocrinol Metab* 24: 1076–1082, 1964. doi:10.1210/jcem-24-10-1076.

6. McIntyre N, Holdsworth CD, Turner DS. New interpretation of oral glucose tolerance. *Lancet* 2: 20–21, 1964. doi:10.1016/s0140-6736(64)90011-x.

7. Brown JC, Mutt V, Pederson RA. Further purification of a polypeptide demonstrating enterogastrone activity. *J Physiol* 209: 57–64, 1970. doi:10.1113/jphysiol.1970.sp009155.

8. Brown JC, Pederson RA, Jorpes E, Mutt V. Preparation of highly active enterogastrone. *Can J Physiol Pharmacol* 47: 113–114, 1969. doi:10.1139/y69-020.

9. Brown JC, Dryburgh JR, Ross SA, Dupre J. Identification and actions of gastric inhibitory polypeptide. *Recent Prog Horm Res* 31: 487–532, 1975. doi:10.1016/b978-0-12-571131-9.50017-7.

10. Dupre J, Ross SA, Watson D, Brown JC. Stimulation of insulin secretion by gastric inhibitory polypeptide in man. *J Clin Endocrinol Metab* 37: 826–828, 1973. doi:10.1210/jcem-37-5-826.

11. Pederson RA, Brown JC. The insulinotropic action of gastric inhibitory polypeptide in the perfused isolated rat pancreas. *Endocrinology* 99: 780–785, 1976. doi:10.1210/endo-99-3-780.

12. Meier JJ, Goetze O, Anstipp J, Hagemann D, Holst JJ, Schmidt WE, Gallwitz B, Nauck MA. Gastric in hibitory polypeptide does not inhibit gastric emptying in humans. *Am J Physiol Endocrinol Metab* 286: E621–E625, 2004. doi:10.1152/ajpendo.00499.2003.

13. Maxwell V, Shulkes A, Brown JC, Solomon TE, Walsh JH, Grossman MI. Effect of gastric inhibitory polypeptide on pentagastrin-stimulated acid secretion in man. *Dig Dis Sci* 25: 113–116, 1980. doi:10.1007/BF01308308.

14. Nauck MA, Bartels E, Orskov C, Ebert R, Creutzfeldt W. Lack of effect of synthetic human gastric inhibitory polypeptide and glucagon-like peptide 1 [7-36 amide] infused at near-physiological concentrations on pentagastrin-stimulated gastric acid secretion in normal human subjects. *Digestion* 52: 214–221, 1992. doi:10.1159/000200956.

15. Brown JC, Pederson RA. *Proceedings of the 5th International Congress of Endocrinology*. Amsterdam, The Netherlands: Excerpta Medica, 1977, p. 568–570.

16. Lund PK, Goodman RH, Dee PC, Habener JF. Pancreatic preproglucagon cDNA contains two glucagon-related coding sequences arranged in tandem. *Proc Natl Acad Sci U S A* 79: 345–349, 1982. doi:10.1073/pnas.79.2.345.

17. Lund PK, Goodman RH, Habener JF. Pancreatic pre-proglucagons are encoded by two separate mRNAs. *J Biol Chem* 256: 6515–6518, 1981.

18. Lund PK, Goodman RH, Montminy MR, Dee PC, Habener JF. Anglerfish islet pre-proglucagon II. Nucleotide and corresponding amino acid sequence of the cDNA. *J Biol Chem* 258: 3280–3284, 1983.

19. Heinrich G, Gros P, Habener JF. Glucagon gene sequence. Four of six exons encode separate functional domains of rat pre-proglucagon. *J Biol Chem* 259: 14082–14087, 1984.

20. Heinrich G, Gros P, Lund PK, Bentley RC, Habener JF. Pre-proglucagon messenger ribonucleic acid: nucleotide and encoded amino acid sequences of the rat pancreatic complementary deoxyribonucleic acid. *Endocrinology* 115: 2176–2181, 1984. doi:10.1210/endo-115-6-2176.

21. Bell GI, Santerre RF, Mullenbach GT. Hamster preproglucagon contains the sequence of glucagon and two related peptides. *Nature* 302: 716–718, 1983. doi:10.1038/302716a0.

22. Bell GI, Sanchez-Pescador R, Laybourn PJ, Najarian RC. Exon duplication and divergence in the human preproglucagon gene. *Nature* 304: 368–371, 1983. doi:10.1038/304368a0.

23. Mojsov S, Heinrich G, Wilson IB, Ravazzola M, Orci L, Habener JF. Preproglucagon gene expression in pancreas and intestine diversifies at the level of post-translational processing. *J Biol Chem* 261: 11880–11889, 1986.

24. Holst JJ, Orskov C, Nielsen OV, Schwartz TW. Truncated glucagon-like peptide I, an insulin-releasing hormone from the distal gut. *FEBS Lett* 211: 169–174, 1987. doi:10.1016/0014-5793(87)81430-8.

25. Mojsov S, Weir GC, Habener JF. Insulinotropin: glucagon-like peptide I (7-37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. *J Clin Invest* 79: 616–619, 1987. doi:10.1172/JCI112855.

26. Drucker DJ, Philippe J, Mojsov S, Chick WL, Habener JF. Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. *Proc Natl Acad Sci U S A* 84: 3434–3438, 1987. doi:10.1073/pnas.84.10.3434.

27. Kreymann B, Williams G, Ghatei MA, Bloom SR. Glucagon-like peptide-1 7-36: a physiological incretin in man. *Lancet* 2: 1300–1304, 1987. doi:10.1016/s0140-6736(87)91194-9.

28. Tseng CC, Zhang XY, Wolfe MM. Effect of GIP and GLP-1 antagonists on insulin release in the rat. *Am J Physiol Endocrinol Physiol* 276: E1049–1054, 1999. doi:10.1152/ajpendo.1999.276.6.E1049.

29. Hansotia T, Baggio LL, Delmeire D, Hinke SA, Yamada Y, Tsukiyama K, Seino Y, Holst JJ, Schuit F, Drucker DJ. Double incretin receptor knockout (DIRKO) mice reveal an essential role for the enteroinsular axis in transducing the glucoregulatory actions of DPP-IV inhibitors. *Diabetes* 53: 1326–1335, 2004. doi:10.2337/diabetes.53.5.1326.

30. Bagger JI, Knop FK, Lund A, Vestergaard H, Holst JJ, Vilsboll T. Impaired regulation of the incretin effect in patients with type 2 diabetes. *J Clin Endocrinol Metab* 96: 737–745, 2011. doi:10.1210/jc.2010-2435.

31. Nauck M, Stockmann F, Ebert R, Creutzfeldt W. Reduced incretin effect in type 2 (non-insulin-dependent) diabetes. *Diabetologia* 29: 46–52, 1986. doi:10.1007/BF02427280.

32. Nauck MA, El-Ouaghlidi A, Gabrys B, Hucking K, Holst JJ, Deacon CF, Gallwitz B, Schmidt WE, Meier JJ. Secretion of incretin hormones (GIP and GLP-1) and incretin effect after oral glucose in first-degree relatives of patients with type 2 diabetes. *Regul Pept* 122: 209–217, 2004. doi:10.1016/j.regpep.2004.06.020.

33. Nauck MA, Homberger E, Siegel EG, Allen RC, Eaton RP, Ebert R, Creutzfeldt W. Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. *J Clin Endocrinol Metab* 63: 492–498, 1986. doi:10.1210/jcem-63-2-492.

34. Perley MJ, Kipnis DM. Plasma insulin responses to oral and intravenous glucose: studies in normal and diabetic sujbjects. *J Clin Invest* 46: 1954–1962, 1967. doi:10.1172/JCI105685.

35. Nauck MA, Heimesaat MM, Orskov C, Holst JJ, Ebert R, Creutzfeldt W. Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. *J Clin Invest* 91: 301–307, 1993. doi:10.1172/JCI116186.

36. Amland PF, Jorde R, Aanderud S, Burhol PG, Giercksky KE. Effects of intravenously infused porcine GIP on serum insulin, plasma C-peptide, and pancreatic polypeptide in non-insulin-dependent diabetes in the fasting state. *Scand J Gastroenterol* 20: 315–320, 1985. doi:10.3109/00365528509091657.

37. Jones IR, Owens DR, Moody AJ, Luzio SD, Morris T, Hayes TM. The effects of glucose-dependent insulinotropic polypeptide infused at physiological concentrations in normal subjects and type 2 (non-insulin-dependent) diabetic patients on glucose tolerance and B-cell secretion. *Diabetologia* 30: 707–712, 1987. doi:10.1007/BF00296993.

38. Krarup T, Saurbrey N, Moody AJ, Kuhl C, Madsbad S. Effect of porcine gastric inhibitory polypeptide on beta-cell function in type I and type II diabetes mellitus. *Metabolism* 36: 677–682, 1987. doi:10.1016/0026-0495(87)90153-3.

39. Meier JJ, Hucking K, Holst JJ, Deacon CF, Schmiegel WH, Nauck MA. Reduced insulinotropic effect of gastric inhibitory polypeptide in first-degree relatives of patients with type 2 diabetes. *Diabetes* 50: 2497–2504, 2001. doi:10.2337/diabetes.50.11.2497.

40. Vilsboll T, Krarup T, Madsbad S, Holst JJ. Defective amplification of the late phase insulin response to glucose by GIP in obese type II diabetic patients. *Diabetologia* 45: 1111–1119, 2002. doi:10.1007/s00125-002-0878-6.

41. Elahi D, McAloon-Dyke M, Fukagawa NK, Meneilly GS, Sclater AL, Minaker KL, Habener JF, Andersen DK. The insulinotropic actions of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (7-37) in normal and diabetic subjects. *Regul Pept* 51: 63–74, 1994. doi:10.1016/0167-0115(94)90136-8.

42. Højberg PV, Vilsbøll T, Rabøl R, Knop FK, Bache M, Krarup T, Holst JJ, Madsbad S. Four weeks of near-normalisation of blood glucose improves the insulin response to glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide in patients with type 2 diabetes. *Diabetologia* 52: 199–207, 2009. doi:10.1007/s00125-008-1195-5.

43. Donahey JC, van Dijk G, Woods SC, Seeley RJ. Intraventricular GLP-1 reduces short- but not long-term food intake or body weight in lean and obese rats. *Brain Res* 779: 75–83, 1998. doi:10.1016/s0006-8993(97)01057-3.

44. Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CM, Meeran K, Choi SJ, Taylor GM, Heath MM, Lambert PD, Wilding JP, Smith DM, Ghatei MA, Herbert J, Bloom SR. A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* 379: 69–72, 1996. doi:10.1038/379069a0.

45. Muller TD, Finan B, Bloom SR, D'Alessio D, Drucker DJ, Flatt PR, Fritsche A, Gribble F, Grill HJ, Habener JF, Holst JJ, Langhans W, Meier JJ, Nauck MA, Perez-Tilve D, Pocai A, Reimann F, Sandoval DA, Schwartz TW, Seeley RJ, Stemmer K, Tang-Christensen M, Woods SC, DiMarchi RD, Tschop MH. Glucagon-like peptide 1 (GLP-1). *Mol Metab* 30: 72–130, 2019. doi:10.1016/j.molmet.2019.09.010.

46. Chen XY, Chen L, Yang W, Xie AM. GLP-1 suppresses feeding behaviors and modulates neuronal electrophysiological properties in multiple brain regions. *Front Mol Neurosci* 14: 793004, 2021. doi:10.3389/fnmol.2021.793004.

47. Geisler CE, Antonellis MP, Trumbauer W, Martin JA, Coskun T, Samms RJ, Hayes MR. Tirzepatide suppresses palatable food intake by selectively reducing preference for fat in rodents. *Diabetes Obes Metab* 25: 56–67, 2023. doi:10.1111/dom.14843.

48. Sisley S, Smith K, Sandoval DA, Seeley RJ. Differences in acute anorectic effects of long-acting GLP-1 receptor agonists in rats. *Peptides* 58: 1–6, 2014. doi:10.1016/j.peptides.2014.05.008.

49. Sisley S, Gutierrez-Aguilar R, Scott M, D'Alessio DA, Sandoval DA, Seeley RJ. Neuronal GLP1R mediates liraglutide's anorectic but not glucose-lowering effect. *J Clin Invest* 124: 2456–2463, 2014. doi:10.1172/JCI72434.

50. Liu J, Pang ZP. Glucagon-like peptide-1 drives energy metabolism on the synaptic highway. *FEBS J* 283: 4413–4423, 2016. doi:10.1111/febs.13785.

51. Rupprecht LE, Mietlicki-Baase EG, Zimmer DJ, McGrath LE, Olivos DR, Hayes MR. Hindbrain GLP-1 receptor-mediated suppression of food intake requires a PI3K-dependent decrease in phosphorylation of membrane-bound Akt. *Am J Physiol Endocrinol Metab* 305: E751–E759, 2013. doi:10.1152/ajpendo.00367.2013.

52. Ohtake N, Saito M, Eto M, Seki K. Exendin-4 promotes the membrane trafficking of the AMPA receptor GluR1 subunit and ADAM10 in the mouse neocortex. *Regul Pept* 190-191: 1–11, 2014. doi:10.1016/j.regpep.2014.04.003.

53. Liu J, Conde K, Zhang P, Lilascharoen V, Xu Z, Lim BK, Seeley RJ, Zhu JJ, Scott MM, Pang ZP. Enhanced AMPA receptor trafficking mediates the anorexigenic effect of endogenous glucagon-like peptide-1 in the paraventricular hypothalamus. *Neuron* 96: 897–909.e895, 2017. doi:10.1016/j.neuron.2017.09.042.

54. Näslund E, Gutniak M, Skogar S, Rössner S, Hellström PM. Glucagon-like peptide 1 increases the period of postprandial satiety and slows gastric emptying in obese men. *Am J Clin Nutr* 68: 525–530, 1998. doi:10.1093/ajcn/68.3.525.

55. Näslund E, Barkeling B, King N, Gutniak M, Blundell JE, Holst JJ, Rössner S, Hellström PM. Energy intake and appetite are suppressed by glucagon-like peptide-1 (GLP-1) in obese men. *Int J Obes Relat Metab Disord* 23: 304–311, 1999. doi:10.1038/sj.ijo.0800818.

56. Gutzwiller JP, Drewe J, Göke B, Schmidt H, Rohrer B, Lareida J, Beglinger C. Glucagon-like peptide-1 promotes satiety and reduces food intake in patients with diabetes mellitus type 2. *Am J Physiol* 276: R1541–1544, 1999. doi:10.1152/ajpregu.1999.276.5.R1541.

57. Mulvihill EE, Varin EM, Gladanac B, Campbell JE, Ussher JR, Baggio LL, Yusta B, Ayala J, Burmeister MA, Matthews D, Bang KW, Ayala JE, Drucker DJ. Cellular sites and mechanisms linking reduction of dipeptidyl peptidase-4 activity to control of incretin hormone action and glucose homeostasis. *Cell Metab* 25: 152–165, 2017. doi:10.1016/j.cmet.2016.10.007.

58. Mentlein R, Gallwitz B, Schmidt WE. Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7-36)amide, peptide histidine methionine and is responsible for their degradation in human serum. *Eur J Biochem* 214: 829–835, 1993. doi:10.1111/j.1432-1033.1993.tb17986.x.

59. Eng J, Kleinman WA, Singh L, Singh G, Raufman JP. Isolation and characterization of exendin-4, an exendin-3 analogue, from Heloderma suspectum venom. Further evidence for an exendin receptor on dispersed acini from guinea pig pancreas. *J Biol Chem* 267: 7402–7405, 1992.

60. Edwards CM, Stanley SA, Davis R, Brynes AE, Frost GS, Seal LJ, Ghatei MA, Bloom SR. Exendin-4 reduces fasting and postprandial glucose and decreases energy intake in healthy volunteers. *Am J Physiol Endocrinol Metab* 281: E155–E161, 2001. doi:10.1152/ajpendo.2001.281.1.E155.

61. Egan JM, Meneilly GS, Elahi D. Effects of 1-mo bolus subcutaneous administration of exendin-4 in type 2 diabetes. *Am J Physiol Endocrinol Metab* 284: E1072–E1079, 2003. doi:10.1152/ajpendo.00315.2002.

62. Poon T, Nelson P, Shen L, Mihm M, Taylor K, Fineman M, Kim D. Exenatide improves glycemic control and reduces body weight in subjects with type 2 diabetes: a dose-ranging study. *Diabetes Technol Ther* 7: 467–477, 2005. doi:10.1089/dia.2005.7.467.

63. Kolterman OG, Buse JB, Fineman MS, Gaines E, Heintz S, Bicsak TA, Taylor K, Kim D, Aisporna M, Wang Y, Baron AD. Synthetic exendin-4 (exenatide) significantly reduces postprandial and fasting plasma glucose in subjects with type 2 diabetes. *J Clin Endocrinol Metab* 88: 3082–3089, 2003. doi:10.1210/jc.2002-021545.

64. DeFronzo RA, Ratner RE, Han J, Kim DD, Fineman MS, Baron AD. Effects of exenatide (exendin-4) on glycemic control and weight over 30 weeks in metformin-treated patients with type 2 diabetes. *Diabetes Care* 28: 1092–1100, 2005. doi:10.2337/diacare.28.5.1092.

65. Muller TD, Clemmensen C, Finan B, DiMarchi RD, Tschop MH. Anti-obesity therapy: from rainbow pills to polyagonists. *Pharmacol Rev* 70: 712–746, 2018. doi:10.1124/pr.117.014803.

66. Agersø H, Jensen LB, Elbrønd B, Rolan P, Zdravkovic M. The pharmacokinetics, pharmacodynamics, safety and tolerability of NN2211, a new long-acting GLP-1 derivative, in healthy men. *Diabetologia* 45: 195–202, 2002. doi:10.1007/s00125-001-0719-z.

67. Feinglos MN, Saad MF, Pi-Sunyer FX, An B, Santiago O, Liraglutide Dose-Response Study Group. Effects of liraglutide (NN2211), a long-acting GLP-1 analogue, on glycaemic control and bodyweight in subjects with type 2 diabetes. *Diabet Med* 22: 1016–1023, 2005. doi:10.1111/j.1464-5491.2005.01567.x.

68. Madsbad S, Schmitz O, Ranstam J, Jakobsen G, Matthews DR, NN2211-1310 International Study Group. Improved glycemic control with no weight increase in patients with type 2 diabetes after once-daily treatment with the long-acting glucagon-like peptide 1 analog liraglutide (NN2211): a 12-week, double-blind, randomized, controlled trial. *Diabetes Care* 27: 1335–1342, 2004. doi:10.2337/diacare.27.6.1335.

69. Madsbad S. Exenatide and liraglutide: different approaches to develop GLP-1 receptor agonists (incretin mimetics)–preclinical and clinical results. *Best Pract Res Clin Endocrinol Metab* 23: 463–477, 2009. doi:10.1016/j.beem.2009.03.008.

70. Pi-Sunyer X, Astrup A, Fujioka K, Greenway F, Halpern A, Krempf M, Lau DC, Le Roux CW, Violante Ortiz R, Jensen CB, Wilding JP, SCALE Obesity and Prediabetes NN8022-1839 Study Group. A randomized, controlled trial of 3.0 mg of liraglutide in weight management. *N Engl J Med* 373: 11–22, 2015. doi:10.1056/NEJMoa1411892.

71. Knudsen LB, Lau J. The discovery and development of liraglutide and semaglutide. *Front Endocrinol (Lausanne)* 10: 155, 2019. doi:10.3389/fendo.2019.00155.

72. Bergmann NC, Davies MJ, Lingvay I, Knop FK. Semaglutide for the treatment of overweight and obesity: a review. *Diabetes Obes Metab* 25: 18–35, 2023. doi:10.1111/dom.14863.

73. Wilding JP, Batterham RL, Calanna S, Davies M, Van Gaal LF, Lingvay I, McGowan BM, Rosenstock J, Tran MT, Wadden TA, Wharton S, Yokote K, Zeuthen N, Kushner RF, STEP 1 Study Group. Once-weekly semaglutide in adults with overweight or obesity. *N Engl J Med* 384: 989–1002, 2021. doi:10.1056/NEJMoa2032183.

74. Rubino D, Abrahamsson N, Davies M, Hesse D, Greenway FL, Jensen C, Lingvay I, Mosenzon O, Rosenstock J, Rubio MA, Rudofsky G, Tadayon S, Wadden TA, Dicker D, STEP 4 Investigators. Effect of continued weekly subcutaneous semaglutide vs placebo on weight loss maintenance in adults with overweight or obesity: the STEP 4 randomized clinical trial. *Jama* 325: 1414–1425, 2021. doi:10.1001/jama.2021.3224.

75. Lincoff AM, Brown-Frandsen K, Colhoun HM, Deanfield J, Emerson SS, Esbjerg S, Hardt-Lindberg S, Hovingh GK, Kahn SE, Kushner RF, Lingvay I, Oral TK, Michelsen MM, Plutzky J, Tornøe CW, Ryan DH, SELECT Trial Investigators. Semaglutide and cardiovascular outcomes in obesity without diabetes. *N Engl J Med* 389: 2221–2232, 2023. doi:10.1056/NEJMoa2307563.

76. Davies M, F×rch L, Jeppesen OK, Pakseresht A, Pedersen SD, Perreault L, Rosenstock J, Shimomura I, Viljoen A, Wadden TA, Lingvay I, STEP 2 Study Group. Semaglutide 2.4 mg once a week in adults with overweight or obesity, and type 2 diabetes (STEP 2): a randomised, double-blind, double-dummy, placebo-controlled, phase 3 trial. *Lancet* 397: 971–984, 2021. doi:10.1016/S0140-6736(21)00213-0.

77. Muller TD, Bluher M, Tschop MH, DiMarchi RD. Anti-obesity drug discovery: advances and challenges. *Nat Rev Drug Discov* 21: 201–223, 2022. doi:10.1038/s41573-021-00337-8.

78. Takahashi Y, Fujita H, Seino Y, Hattori S, Hidaka S, Miyakawa T, Suzuki A, Waki H, Yabe D, Seino Y, Yamada Y. Gastric inhibitory polypeptide receptor antagonism suppresses intramuscular adipose tissue accumulation and ameliorates sarcopenia. *J Cachexia Sarcopenia Muscle* 14: 2703–2718, 2023. doi:10.1002/jcsm.13346.

79. Yamada C, Yamada Y, Tsukiyama K, Yamada K, Yamane S, Harada N, Miyawaki K, Seino Y, Inagaki N. Genetic inactivation of GIP signaling reverses aging-associated insulin resistance through body composition changes. *Biochem Biophys Res Commun* 364: 175–180, 2007. doi:10.1016/j.bbrc.2007.09.128.

80. Boer GA, Keenan SN, Miotto PM, Holst JJ, Watt MJ. GIP receptor deletion in mice confers resistance to high-fat diet-induced obesity via alterations in energy expenditure and adipose tissue lipid metabolism. *Am J Physiol Endocrinol Metab* 320: E835–E845, 2021. doi:10.1152/ajpendo.00646.2020.

81. Miyawaki K, Yamada Y, Ban N, Ihara Y, Tsukiyama K, Zhou H, Fujimoto S, Oku A, Tsuda K, Toyokuni S, Hiai H, Mizunoya W, Fushiki T, Holst JJ, Makino M, Tashita A, Kobara Y, Tsubamoto Y, Jinnouchi T, Jomori T, Seino Y. Inhibition of gastric inhibitory polypeptide signaling prevents obesity. *Nat Med* 8: 738–742, 2002. doi:10.1038/nm727.

82. Eckel RH, Fujimoto WY, Brunzell JD. Gastric inhibitory polypeptide enhanced lipoprotein lipase activity in cultured preadipocytes. *Diabetes* 28: 1141–1142, 1979. doi:10.2337/diab.28.12.1141.

83. Kim SJ, Nian C, McIntosh CH. Activation of lipoprotein lipase by glucose-dependent insulinotropic polypeptide in adipocytes. A role for a protein kinase B, LKB1, and AMP-activated protein kinase cascade. *J Biol Chem* 282: 8557–8567, 2007. doi:10.1074/jbc.M609088200.

84. Kim SJ, Nian C, McIntosh CH. Resistin is a key mediator of glucose-dependent insulinotropic polypeptide (GIP) stimulation of lipoprotein lipase (LPL) activity in adipocytes. *J Biol Chem* 282: 34139–34147, 2007. doi:10.1074/jbc.M704896200.

85. Hauner H, Glatting G, Kaminska D, Pfeiffer EF. Effects of gastric inhibitory polypeptide on glucose and lipid metabolism of isolated rat adipocytes. *Ann Nutr Metab* 32: 282–288, 1988. doi:10.1159/000177467.

86. Starich GH, Bar RS, Mazzaferri EL. GIP increases insulin receptor affinity and cellular sensitivity in adipocytes. *Am J Physiol Endocrinol Metab* 249: E603–E607, 1985. doi:10.1152/ajpendo.1985.249.6.E603.

87. Song DH, Getty-Kaushik L, Tseng E, Simon J, Corkey BE, Wolfe MM. Glucose-dependent insulinotropic polypeptide enhances adipocyte development and glucose uptake in part through Akt activation. *Gastroenterology* 133: 1796–1805, 2007. doi:10.1053/j.gastro.2007.09.005.

88. Kizilkaya HS, Sorensen KV, Kibsgaard CJ, Gasbjerg LS, Hauser AS, Sparre-Ulrich AH, Grarup N, Rosenkilde MM. Loss of function glucose-dependent insulinotropic polypeptide receptor variants are associated with alterations in BMI, bone strength and cardiovascular outcomes. *Front Cell Dev Biol* 9: 749607, 2021. doi:10.3389/fcell.2021.749607.

89. Speliotes EK, Willer CJ, Berndt SI, Monda KL, Thorleifsson G, Jackson AU, et al. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nat Genet* 42: 937–948, 2010. doi:10.1038/ng.686.

90. Vogel CI, Scherag A, Bronner G, Nguyen TT, Wang HJ, Grallert H, Bornhorst A, Rosskopf D, Volzke H, Reinehr T, Rief W, Illig T, Wichmann HE, Schafer H, Hebebrand J, Hinney A. Gastric inhibitory polypeptide receptor: association analyses for obesity of several polymorphisms in large study groups. *BMC Med Genet* 10: 19, 2009. doi:10.1186/1471-2350-10-19.

91. Killion EA, Wang J, Yie J, Shi SD, Bates D, Min X, Komorowski R, Hager T, Deng L, Atangan L, Lu SC, Kurzeja RJ, Sivits G, Lin J, Chen Q, Wang Z, Thibault SA, Abbott CM, Meng T, Clavette B, Murawsky CM, Foltz IN, Rottman JB, Hale C, Veniant MM, Lloyd DJ. Anti-obesity effects of GIPR antagonists alone and in combination with GLP-1R agonists in preclinical models. *Sci Transl Med* 10: eaat3392, 2018. doi:10.1126/scitranslmed.aat3392.

92. Yang B, Gelfanov VM, El K, Chen A, Rohlfs R, DuBois B, Kruse Hansen AM, Perez-Tilve D, Knerr PJ, D'Alessio D, Campbell JE, Douros JD, Finan B. Discovery of a potent GIPR peptide antagonist that is effective in rodent and human systems. *Mol Metab* 66: 101638, 2022. doi:10.1016/j.molmet.2022.101638.

93. Ebert R, Creutzfeldt W. Metabolic effects of gastric inhibitory polypeptide. *Front Horm Res* 16: 175–185, 1987.

94. McIntosh CH, Bremsak I, Lynn FC, Gill R, Hinke SA, Gelling R, Nian C, McKnight G, Jaspers S, Pederson RA. Glucose-dependent insulinotropic polypeptide stimulation of lipolysis in differentiated 3T3-L1 cells: wortmannin-sensitive inhibition by insulin. *Endocrinology* 140: 398–404, 1999. doi:10.1210/endo.140.1.6464.

95. Heimburger SM, Nielsen CN, Calanna S, Holst JJ, Vilsboll T, Knop FK, Christensen MB. Glucose-dependent insulinotropic polypeptide induces lipolysis during stable basal insulin substitution and hyperglycaemia in men with type 1 diabetes: a randomized, double-blind, placebo-controlled, crossover clinical trial. *Diabetes Obes Metab* 24: 142–147, 2022. doi:10.1111/dom.14545.

96. Hinke SA, Pauly RP, Ehses J, Kerridge P, Demuth HU, McIntosh CH, Pederson RA. Role of glucose in chronic desensitization of isolated rat islets and mouse insulinoma (betaTC-3) cells to glucose-dependent insulinotropic polypeptide. *J Endocrinol* 165: 281–291, 2000. doi:10.1677/joe.0.1650281.

97. Amiranoff B, Vauclin-Jacques N, Laburthe M. Functional GIP receptors in a hamster pancreatic beta cell line, In 111: specific binding and biological effects. *Biochem Biophys Res Commun* 123: 671–676, 1984. doi:10.1016/0006-291x(84)90281-x.

98. Wang L, Pydi SP, Cui Y, Zhu L, Meister J, Gavrilova O, Berdeaux R, Fortin JP, Bence KK, Vernochet C, Wess J. Selective activation of G(s) signaling in adipocytes causes striking metabolic improvements in mice. *Mol Metab* 27: 83–91, 2019. doi:10.1016/j.molmet.2019.06.018.

99. Zhang Q, Delessa CT, Augustin R, Bakhti M, Collden G, Drucker DJ, et al. The glucose-dependent insulinotropic polypeptide (GIP) regulates body weight and food intake via CNS-GIPR signaling. *Cell Metab* 33: 833–844 e835, 2021. doi:10.1016/j.cmet.2021.01.015.

100. Kim SJ, Nian C, Karunakaran S, Clee SM, Isales CM, McIntosh CH. GIP-overexpressing mice demonstrate reduced diet-induced obesity and steatosis, and improved glucose homeostasis. *PLoS One* 7: e40156, 2012. doi:10.1371/journal.pone.0040156.

101. Joo E, Harada N, Yamane S, Fukushima T, Taura D, Iwasaki K, Sankoda A, Shibue K, Harada T, Suzuki K, Hamasaki A, Inagaki N. Inhibition of gastric inhibitory polypeptide receptor signaling in adipose tissue reduces insulin resistance and hepatic steatosis in high-fat diet-fed mice. *Diabetes* 66: 868–879, 2017. doi:10.2337/db16-0758.

102. Campbell JE, Beaudry JL, Svendsen B, Baggio LL, Gordon AN, Ussher JR, Wong CK, Gribble FM, D'Alessio DA, Reimann F, Drucker DJ. GIPR is predominantly localized to nonadipocyte cell types within white adipose tissue. *Diabetes* 71: 1115–1127, 2022. doi:10.2337/db21-1166.

103. Killion EA, Chen M, Falsey JR, Sivits G, Hager T, Atangan L, Helmering J, Lee J, Li H, Wu B, Cheng Y, Veniant MM, Lloyd DJ. Chronic glucose-dependent insulinotropic polypeptide receptor (GIPR) agonism desensitizes adipocyte GIPR activity mimicking functional GIPR antagonism. *Nat Commun* 11: 4981, 2020. doi:10.1038/s41467-020-18751-8.

104. Longden TA, Zhao G, Hariharan A, Lederer WJ. Pericytes and the control of blood flow in brain and heart. *Annu Rev Physiol* 85: 137–164, 2023. doi:10.1146/annurev-physiol-031522-034807.

105. Wu Y, Fu J, Huang Y, Duan R, Zhang W, Wang C, Wang S, Hu X, Zhao H, Wang L, Liu J, Gao G, Yuan P. Biology and function of pericytes in the vascular microcirculation. *Animal Model Exp Med* 6: 337–345, 2023. doi:10.1002/ame2.12334.

106. Asmar M, Asmar A, Simonsen L, Gasbjerg LS, Sparre-Ulrich AH, Rosenkilde MM, Hartmann B, Dela F, Holst JJ, Bulow J. The gluco- and liporegulatory and vasodilatory effects of glucose-dependent insulinotropic polypeptide (GIP) are abolished by an antagonist of the human GIP receptor. *Diabetes* 66: 2363–2371, 2017. doi:10.2337/db17-0480.

107. Asmar M, Simonsen L, Madsbad S, Stallknecht B, Holst JJ, Bulow J. Glucose-dependent insulinotropic polypeptide may enhance fatty acid re-esterification in subcutaneous abdominal adipose tissue in lean humans. *Diabetes* 59: 2160–2163, 2010. doi:10.2337/db10-0098.

108. Kim SJ, Nian C, McIntosh CH. GIP increases human adipocyte LPL expression through CREB and TORC2-mediated trans-activation of the LPL gene. *J Lipid Res* 51: 3145–3157, 2010. doi:10.1194/jlr.M006841.

109. Knapper JM, Puddicombe SM, Morgan LM, Fletcher JM. Investigations into the actions of glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1(7-36)amide on lipoprotein lipase activity in explants of rat adipose tissue. *J Nutr* 125: 183–188, 1995. doi:10.1093/jn/125.2.183.

110. Liskiewicz A, Khalil A, Liskiewicz D, Novikoff A, Grandl G, Maity-Kumar G, et al. Glucose-dependent insulinotropic polypeptide regulates body weight and food intake via GABAergic neurons in mice. *Nat Metab* 5: 2075–2085, 2023. doi:10.1038/s42255-023-00931-7.

111. Mantelmacher FD, Zvibel I, Cohen K, Epshtein A, Pasmanik-Chor M, Vogl T, Kuperman Y, Weiss S, Drucker DJ, Varol C, Fishman S. GIP regulates inflammation and body weight by restraining myeloid-cell-derived S100A8/A9. *Nat Metab* 1: 58–69, 2019. doi:10.1038/s42255-018-0001-z.

112. Campbell JE, Ussher JR, Mulvihill EE, Kolic J, Baggio LL, Cao X, Liu Y, Lamont BJ, Morii T, Streutker CJ, Tamarina N, Philipson LH, Wrana JL, MacDonald PE, Drucker DJ. TCF1 links GIPR signaling to the control of beta cell function and survival. *Nat Med* 22: 84–90, 2016. doi:10.1038/nm.3997.

113. Killion EA, Wang J, Yie J, Shi SD, Bates D, Min X, Komorowski R, Hager T, Deng L, Atangan L, Lu SC, Kurzeja RJ, Sivits G, Lin J, Chen Q, Wang Z, Thibault SA, Abbott CM, Meng T, Clavette B, Murawsky CM, Foltz IN, Rottman JB, Hale C, Véniant MM, Lloyd DJ. Anti-obesity effects of GIPR antagonists alone and in combination with GLP-1R agonists in preclinical models. *Sci Transl Med* 10, 2018. doi:10.1126/scitranslmed.aat3392.

114. Beaudry JL, Kaur KD, Varin EM, Baggio LL, Cao X, Mulvihill EE, Stern JH, Campbell JE, Scherer PE, Drucker DJ. The brown adipose tissue glucagon receptor is functional but not essential for control of energy homeostasis in mice. *Mol Metab* 22: 37–48, 2019. doi:10.1016/j.molmet.2019.01.011.

115. Adriaenssens AE, Biggs EK, Darwish T, Tadross J, Sukthankar T, Girish M, Polex-Wolf J, Lam BY, Zvetkova I, Pan W, Chiarugi D, Yeo GS, Blouet C, Gribble FM, Reimann F. Glucose-dependent insulinotropic polypeptide receptor-expressing cells in the hypothalamus regulate food intake. *Cell Metab* 30: 987–996, 2019. doi:10.1016/j.cmet.2019.07.013.

116. Adriaenssens A, Broichhagen J, de Bray A, Ast J, Hasib A, Jones B, Tomas A, Burgos NF, Woodward O, Lewis J, O'Flaherty E, El K, Cui C, Harada N, Inagaki N, Campbell J, Brierley D, Hodson DJ, Samms R, Gribble F, Reimann F. Hypothalamic and brainstem glucose-dependent insulinotropic polypeptide receptor neurons employ distinct mechanisms to affect feeding. *JCI Insight* 8, 2023. doi:10.1172/jci.insight.164921.

117. Holst JJ, Rosenkilde MM. GIP as a therapeutic target in diabetes and obesity: insight from incretin co-agonists. *J Clin Endocrinol Metab* 105: e2710–e2716, 2020. doi:10.1210/clinem/dgaa327.

118. Baggio LL, Kim JG, Drucker DJ. Chronic exposure to GLP-1R agonists promotes homologous GLP-1 receptor desensitization in vitro but does not attenuate GLP-1R-dependent glucose homeostasis in vivo. *Diabetes* 53, Suppl 3: S205–S214, 2004. doi:10.2337/diabetes.53.suppl_3.s205.

119. Fehmann HC, Habener JF. Homologous desensitization of the insulinotropic glucagon-like peptide-I (7-37) receptor on insulinoma (HIT-T15) cells. *Endocrinology* 128: 2880–2888, 1991. doi:10.1210/endo-128-6-2880.

120. Tschop MH, Friedman JM. Seeking satiety: from signals to solutions. *Sci Transl Med* 15: eadh4453, 2023. doi:10.1126/scitranslmed.adh4453.

121. Mroz PA, Finan B, Gelfanov V, Yang B, Tschop MH, DiMarchi RD, Perez-Tilve D. Optimized GIP analogs promote body weight lowering in mice through GIPR agonism not antagonism. *Mol Metab* 20: 51–62, 2019. doi:10.1016/j.molmet.2018.12.001.

122. Iwasaki A. Immune regulation of antibody access to neuronal tissues. *Trends Mol Med* 23: 227–245, 2017. doi:10.1016/j.molmed.2017.01.004.

123. Kaneko K, Fu Y, Lin HY, Cordonier EL, Mo Q, Gao Y, Yao T, Naylor J, Howard V, Saito K, Xu P, Chen SS, Chen MH, Xu Y, Williams KW, Ravn P, Fukuda M. Gut-derived GIP activates central Rap1 to impair neural leptin sensitivity during overnutrition. *J Clin Invest* 129: 3786–3791, 2019. doi:10.1172/JCI126107.

124. Sparre-Ulrich AH, Gabe MN, Gasbjerg LS, Christiansen CB, Svendsen B, Hartmann B, Holst JJ, Rosenkilde MM. GIP(3-30)NH(2) is a potent competitive antagonist of the GIP receptor and effectively inhibits GIP-mediated insulin, glucagon, and somatostatin release. *Biochem Pharmacol* 131: 78–88, 2017. doi:10.1016/j.bcp.2017.02.012.

125. El K, Douros JD, Willard FS, Novikoff A, Sargsyan A, Perez-Tilve D, Wainscott DB, Yang B, Chen A, Wothe D, Coupland C, Tschop MH, Finan B, D'Alessio DA, Sloop KW, Muller TD, Campbell JE. The incretin co-agonist tirzepatide requires GIPR for hormone secretion from human islets. *Nat Metab* 5: 945–954, 2023. doi:10.1038/s42255-023-00811-0.

126. Samms RJ, Christe ME, Collins KA, Pirro V, Droz BA, Holland AK, Friedrich JL, Wojnicki S, Konkol DL, Cosgrove R, Furber EP, Ruan X, O'Farrell LS, Long AM, Dogra M, Willency JA, Lin Y, Ding L, Cheng CC, Cabrera O, Briere DA, Alsina-Fernandez J, Gimeno RE, Moyers JS, Coskun T, Coghlan MP, Sloop KW, Roell WC. GIPR agonism mediates weight-independent insulin sensitization by tirzepatide in obese mice. *J Clin Invest* 131: e146353, 2021. doi:10.1172/JCI146353.

127. Finan B, Ma T, Ottaway N, Muller TD, Habegger KM, Heppner KM, Kirchner H, Holland J, Hembree J, Raver C, Lockie SH, Smiley DL, Gelfanov V, Yang B, Hofmann S, Bruemmer D, Drucker DJ, Pfluger PT, Perez-Tilve D, Gidda J, Vignati L, Zhang L, Hauptman JB, Lau M, Brecheisen M, Uhles S, Riboulet W, Hainaut E, Sebokova E, Conde-Knape K, Konkar A, DiMarchi RD, Tschöp MH. Unimolecular dual incretins maximize metabolic benefits in rodents, monkeys, and humans. *Sci Transl Med* 5: 209ra151, 2013. doi:10.1126/scitranslmed.3007218.

128. Coskun T, Sloop KW, Loghin C, Alsina-Fernandez J, Urva S, Bokvist KB, Cui X, Briere DA, Cabrera O, Roell WC, Kuchibhotla U, Moyers JS, Benson CT, Gimeno RE, D'Alessio DA, Haupt A. LY3298176, a novel dual GIP and GLP-1 receptor agonist for the treatment of type 2 diabetes mellitus: From discovery to clinical proof of concept. *Mol Metab* 18: 3–14, 2018. doi:10.1016/j.molmet.2018.09.009.

129. NamKoong C, Kim MS, Jang BT, Lee YH, Cho YM, Choi HJ. Central administration of GLP-1 and GIP decreases feeding in mice. *Biochem Biophys Res Commun* 490: 247–252, 2017. doi:10.1016/j.bbrc.2017.06.031.

130. Skovbjerg G, Roostalu U, Salinas CG, Skytte JL, Perens J, Clemmensen C, Elster L, Frich CK, Hansen HH, Hecksher-Sørensen J. Uncovering CNS access of lipidated exendin-4 analogues by quantitative whole-brain 3D light sheet imaging. *Neuropharmacology* 238: 109637, 2023. doi:10.1016/j.neuropharm.2023.109637.

131. Salameh TS, Rhea EM, Talbot K, Banks WA. Brain uptake pharmacokinetics of incretin receptor agonists showing promise as Alzheimer's and Parkinson's disease therapeutics. *Biochem Pharmacol* 210: 115474, 2020. doi:10.1016/j.bcp.2023.115474.

132. Salinas CB, Lu TT, Gabery S, Marstal K, Alanentalo T, Mercer AJ, Cornea A, Conradsen K, Hecksher-Sorensen J, Dahl AB, Knudsen LB, Secher A. Integrated brain atlas for unbiased mapping of nervous system effects following liraglutide treatment. *Sci Rep* 8: 10310, 2018. doi:10.1038/s41598-018-28496-6.

133. Gabery S, Salinas CG, Paulsen SJ, Ahnfelt-Ronne J, Alanentalo T, Baquero AF, Buckley ST, Farkas E, Fekete C, Frederiksen KS, Helms HC, Jeppesen JF, John LM, Pyke C, Nohr J, Lu TT, Polex-Wolf J, Prevot V, Raun K, Simonsen L, Sun G, Szilvasy-Szabo A, Willenbrock H, Secher A, Knudsen LB, Hogendorf WF. Semaglutide lowers body weight in rodents via distributed neural pathways. *JCI Insight* 5: e133429, 2020. doi:10.1172/jci.insight.133429.

134. Costa A, Ai M, Nunn N, Culotta I, Hunter J, Boudjadja MB, Valencia-Torres L, Aviello G, Hodson DJ, Snider BM, Coskun T, Emmerson PJ, Luckman SM, D'Agostino G. Anorectic and aversive effects of GLP-1 receptor agonism are mediated by brainstem cholecystokinin neurons, and modulated by GIP receptor activation. *Mol Metab* 55: 101407, 2022. doi:10.1016/j.molmet.2021.101407.

135. Sikirica MV, Martin AA, Wood R, Leith A, Piercy J, Higgins V. Reasons for discontinuation of GLP1 receptor agonists: data from a real-world cross-sectional survey of physicians and their patients with type 2 diabetes. *Diabetes Metab Syndr Obes* 10: 403–412, 2017. doi:10.2147/DMSO.S141235.

136. Zhang Z, Zhang Q, Tan Y, Chen Y, Zhou X, Liu S, Yu J. GLP-1RAs caused gastrointestinal adverse reactions of drug withdrawal: a system review and network meta-analysis. *Front Endocrinol (Lausanne)* 14: 1149328, 2023. doi:10.3389/fendo.2023.1149328.

137. Petri KC, Ingwersen SH, Flint A, Zacho J, Overgaard RV. Exposure-response analysis for evaluation of semaglutide dose levels in type 2 diabetes. *Diabetes Obes Metab* 20: 2238–2245, 2018. doi:10.1111/dom.13358.

138. Davies M, Pieber TR, Hartoft-Nielsen ML, Hansen OK, Jabbour S, Rosenstock J. Effect of oral semaglutide compared with placebo and subcutaneous semaglutide on glycemic control in patients with type 2 diabetes: a randomized clinical trial. *JAMA* 318: 1460–1470, 2017. doi:10.1001/jama.2017.14752.

139. O'Neil PM, Birkenfeld AL, McGowan B, Mosenzon O, Pedersen SD, Wharton S, Carson CG, Jepsen CH, Kabisch M, Wilding JP. Efficacy and safety of semaglutide compared with liraglutide and placebo for weight loss in patients with obesity: a randomised, double-blind, placebo and active controlled, dose-ranging, phase 2 trial. *Lancet* 392: 637–649, 2018. doi:10.1016/S0140-6736(18)31773-2.

140. Borner T, Geisler CE, Fortin SM, Cosgrove R, Alsina-Fernandez J, Dogra M, Doebley S, Sanchez-Navarro MJ, Leon RM, Gaisinsky J, White A, Bamezai A, Ghidewon MY, Grill HJ, Crist RC, Reiner BC, Ai M, Samms RJ, De Jonghe BC, Hayes MR. GIP receptor agonism attenuates GLP-1 receptor agonist-induced nausea and emesis in preclinical models. *Diabetes* 70: 2545–2553, 2021. doi:10.2337/db21-0459.

141. Garvey WT, Frias JP, Jastreboff AM, Le Roux CW, Sattar N, Aizenberg D, Mao H, Zhang S, Ahmad NN, Bunck MC, Benabbad I, Zhang XM, SURMOUNT-2 investigators. Tirzepatide once weekly for the treatment of obesity in people with type 2 diabetes (SURMOUNT-2): a double-blind, randomised, multicentre, placebo-controlled, phase 3 trial. *Lancet* 402: 613–626, 2023. doi:10.1016/S0140-6736(23)01200-X.

142. Novikoff A, O'Brien SL, Bernecker M, Grandl G, Kleinert M, Knerr PJ, Stemmer K, Klingenspor M, Zeigerer A, DiMarchi R, Tschop MH, Finan B, Calebiro D, Muller TD. Spatiotemporal GLP-1 and GIP receptor signaling and trafficking/recycling dynamics induced by selected receptor mono- and dual-agonists. *Mol Metab* 49: 101181, 2021. doi:10.1016/j.molmet.2021.101181.

143. Willard FS, Douros JD, Gabe MB, Showalter AD, Wainscott DB, Suter TM, Capozzi ME, van der Velden WJC, Stutsman C, Cardona GR, Urva S, Emmerson PJ, Holst JJ, D'Alessio DA, Coghlan MP, Rosenkilde MM, Campbell JE, Sloop KW. Tirzepatide is an imbalanced and biased dual GIP and GLP-1 receptor agonist. *JCI Insight* 5: e140532, 2020. doi:10.1172/jci.insight.140532.

144. Yuliantie E, Darbalaei S, Dai A, Zhao P, Yang D, Sexton PM, Wang MW, Wootten D. Pharmacological characterization of mono-, dual- and tri-peptidic agonists at GIP and GLP-1 receptors. *Biochem Pharmacol* 177: 114001, 2020. doi:10.1016/j.bcp.2020.114001.

145. Zhang C, Kaye JA, Cai Z, Wang Y, Prescott SL, Liberles SD. Area postrema cell types that mediate nausea-associated behaviors. *Neuron* 109: 461–472.e465, 2021. doi:10.1016/j.neuron.2020.11.010.

146. Ludwig MQ, Cheng W, Gordian D, Lee J, Paulsen SJ, Hansen SN, Egerod KL, Barkholt P, Rhodes CJ, Secher A, Knudsen LB, Pyke C, Myers MG Jr, Pers TH. A genetic map of the mouse dorsal vagal complex and its role in obesity. *Nat Metab* 3: 530–545, 2021. doi:10.1038/s42255-021-00363-1.

147. Del Prato S, Kahn SE, Pavo I, Weerakkody GJ, Yang Z, Doupis J, Aizenberg D, Wynne AG, Riesmeyer JS, Heine RJ, Wiese RJ, SURPASS-4 Investigators. Tirzepatide versus insulin glargine in type 2 diabetes and increased cardiovascular risk (SURPASS-4): a randomised, open-label, parallel-group, multicentre, phase 3 trial. *Lancet* 398: 1811–1824, 2021. doi:10.1016/S0140-6736(21)02188-7.

148. Frias JP, Davies MJ, Rosenstock J, Perez Manghi FC, Fernandez Lando L, Bergman BK, Liu B, Cui X, Brown K, SURPASS-4 Investigators. Tirzepatide versus semaglutide once weekly in patients with type 2 diabetes. *N Engl J Med* 385: 503–515, 2021. doi:10.1056/NEJMoa2107519.

149. Ludvik B, Giorgino F, Jodar E, Frias JP, Fernandez Lando L, Brown K, Bray R, Rodriguez A. Once-weekly tirzepatide versus once-daily insulin degludec as add-on to metformin with or without SGLT2 inhibitors in patients with type 2 diabetes (SURPASS-3): a randomised, open-label, parallel-group, phase 3 trial. *Lancet* 398: 583–598, 2021. doi:10.1016/S0140-6736(21)01443-4.

150. Rosenstock J, Wysham C, Frias JP, Kaneko S, Lee CJ, Fernandez Lando L, Mao H, Cui X, Karanikas CA, Thieu VT. Efficacy and safety of a novel dual GIP and GLP-1 receptor agonist tirzepatide in patients with type 2 diabetes (SURPASS-1): a double-blind, randomised, phase 3 trial. *Lancet* 398: 143–155, 2021. doi:10.1016/S0140-6736(21)01324-6.

151. Rodriguez PJ, Goodwin Cartwright BM, Gratzl S, Baker C, Gluckman TJ, Stucky NL. Comparative effectiveness of semaglutide and tirzepatide for weight loss in adults with overweight and obesity in the US: a real-world evidence study (Preprint). *medRxiv* 2023.11.21.23298775, 2023.

152. Jastreboff AM, Aronne LJ, Ahmad NN, Wharton S, Connery L, Alves B, Kiyosue A, Zhang S, Liu B, Bunck MC, Stefanski A, SURMOUNT-1 Investigators. Tirzepatide once weekly for the treatment of obesity. *N Engl J Med* 387: 205–216, 2022. doi:10.1056/NEJMoa2206038.

153. Eli Lilly. Tirzepatide Demonstrated Significant and Superior Weight Loss Compared to Placebo in Two Pivotal Studies. 2023. https://investor.lilly.com/news-releases/news-release-details/tirzepatide-demonstrated-significant-and-superior-weight-loss.

154. Dahl D, Onishi Y, Norwood P, Huh R, Bray R, Patel H, Rodríguez Á. Effect of subcutaneous tirzepatide vs placebo added to titrated insulin glargine on glycemic control in patients with type 2 diabetes: the SURPASS-5 Randomized Clinical Trial. *JAMA* 327: 534–545, 2022. doi:10.1001/jama.2022.0078.

155. Campbell JE. Targeting the GIPR for obesity: to agonize or antagonize? Potential mechanisms. *Mol Metab* 46: 101139, 2021. doi:10.1016/j.molmet.2020.101139.

156. Lu SC, Chen M, Atangan L, Killion EA, Komorowski R, Cheng Y, Netirojjanakul C, Falsey JR, Stolina M, Dwyer D, Hale C, Stanislaus S, Hager T, Thomas VA, Harrold JM, Lloyd DJ, Véniant MM. GIPR antagonist antibodies conjugated to GLP-1 peptide are bispecific molecules that decrease weight in obese mice and monkeys. *Cell Rep Med* 2: 100263, 2021. doi:10.1016/j.xcrm.2021.100263.

157. Al-Zaid B, Chacko S, Ezeamuzie CI, Bünemann M, Krasel C, Karimian T, Lanzerstorfer P, Al-Sabah S. Differential effects of glucose-dependent insulinotropic polypeptide receptor/glucagon-like peptide-1 receptor heteromerization on cell signaling when expressed in HEK-293 cells. *Pharmacol Res Perspect* 10: e01013, 2022. doi:10.1002/prp2.1013.

158. Amgen. Amgen Presents New AMG 133 Phase 1 Clinical Data at WCIRDC 2022. 2023. https://www.amgen.com/newsroom/press-releases/2022/12/amgen-presents-new-amg-133-phase-1-clinical-data-at-wcirdc-2022.

159. Jones B, Buenaventura T, Kanda N, Chabosseau P, Owen BM, Scott R, Goldin R, Angkathunyakul N, Corrêa IR, Bosco D, Johnson PR, Piemonti L, Marchetti P, Shapiro AM, Cochran BJ, Hanyaloglu AC, Inoue A, Tan T, Rutter GA, Tomas A, Bloom SR. Targeting GLP-1 receptor trafficking to improve agonist efficacy. *Nat Commun* 9: 1602, 2018. doi:10.1038/s41467-018-03941-2.

160. Lucey M, Pickford P, Bitsi S, Minnion J, Ungewiss J, Schoeneberg K, Rutter GA, Bloom SR, Tomas A, Jones B. Disconnect between signalling potency and in vivo efficacy of pharmacokinetically optimised biased glucagon-like peptide-1 receptor agonists. *Mol Metab* 37: 100991, 2020. doi:10.1016/j.molmet.2020.100991.

161. Bitsi S, El Eid L, Manchanda Y, Oqua AI, Mohamed N, Hansen B, Suba K, Rutter GA, Salem V, Jones B, Tomas A. Divergent acute versus prolonged pharmacological GLP-1R responses in adult β cell-specific β-arrestin 2 knockout mice. *Sci Adv* 9: eadf7737, 2023. doi:10.1126/sciadv.adf7737.

162. Hinds CE, Peace E, Chen S, Davies I, El Eid L, Tomas A, Tan T, Minnion J, Jones B, Bloom SR. Abolishing β-arrestin recruitment is necessary for the full metabolic benefits of G protein-biased glucagon-like peptide-1 receptor agonists. *Diabetes Obes Metab* 26: 65–77, 2023. doi:10.1111/dom.15288.

163. Coghlan MP, O'Farrell L, Showalter AD, Wainscott DB, Stutsman C, Cardona G, Cabrera O, Alsina-Fernandez J, Willard FS, Sloop K, Coskun T. 639-P: GIP receptor agonism enhances weight loss from either a biased or an unbiased GLP-1 receptor agonist in DIO mice. *Diabetes* 70: 639-P, 2021. doi:10.2337/db21-639-P.

164. Rajagopal S, Shenoy SK. GPCR desensitization: acute and prolonged phases. *Cell Signal* 41: 9–16, 2018. doi:10.1016/j.cellsig.2017.01.024.

165. Jones B, McGlone ER, Fang Z, Pickford P, Corrêa IR Jr, Oishi A, Jockers R, Inoue A, Kumar S, Görlitz F, Dunsby C, French PMW. Rutter GA, Tan T, Tomas A, Bloom SR. Genetic and biased agonist-mediated reductions in β-arrestin recruitment prolong cAMP signaling at glucagon family receptors. *J Biol Chem* 296: 100133, 2021. doi:10.1074/jbc.RA120.016334.

166. Manchanda Y, Bitsi S, Chen S, Broichhagen J, Bernardino de la Serna J, Jones B, Tomas A. Enhanced endosomal signaling and desensitization of GLP-1R vs GIPR in pancreatic beta cells. *Endocrinology* 164: bqad028, 2023. doi:10.1210/endocr/bqad028.

167. Mohammad S, Patel RT, Bruno J, Panhwar MS, Wen J, McGraw TE. A naturally occurring GIP receptor variant undergoes enhanced agonist-induced desensitization, which impairs GIP control of adipose insulin sensitivity. *Mol Cell Biol* 34: 3618–3629, 2014. doi:10.1128/MCB.00256-14.

168. Gabe MB, van der Velden WJ, Gadgaard S, Smit FX, Hartmann B, Bräuner-Osborne H, Rosenkilde MM. Enhanced agonist residence time, internalization rate and signalling of the GIP receptor variant [E354Q] facilitate receptor desensitization and long-term impairment of the GIP system. *Basic Clin Pharmacol Toxicol* 126, Suppl 6: 122–132, 2020. doi:10.1111/bcpt.13289.

169. Oduori OS, Murao N, Shimomura K, Takahashi H, Zhang Q, Dou H, Sakai S, Minami K, Chanclon B, Guida C, Kothegala L, Tolö J, Maejima Y, Yokoi N, Minami Y, Miki T, Rorsman P, Seino S. Gs/Gq signaling switch in β cells defines incretin effectiveness in diabetes. *J Clin Invest* 130: 6639–6655, 2020. doi:10.1172/JCI140046.