Glucagon-like peptide-1 (GLP-1) receptor agonists improve glucose homeostasis, reduce body weight, and over time benefit cardiovascular health in type 2 diabetes mellitus (T2DM). However, dose-related gastrointestinal effects limit efficacy, and therefore agents possessing GLP-1 pharmacology that can also target alternative pathways may expand the therapeutic index. One approach is to engineer GLP-1 activity into the sequence of glucose-dependent insulinotropic polypeptide (GIP). Although the therapeutic implications of the lipogenic actions of GIP are debated, its ability to improve lipid and glucose metabolism is especially evident when paired with the anorexigenic mechanism of GLP-1. We review the complexity of GIP in regulating adipose tissue function and energy balance in the context of recent findings in T2DM showing that dual GIP/GLP-1 receptor agonist therapy produces profound weight loss, glycemic control, and lipid lowering.

GLP-1 and Beyond
While the discoveries of insulin and glucagon during the 20th century provided our foundational understanding of glucose homeostasis [1,2], the pivot toward the new millennium elucidated sophisticated mechanisms whereby the incretins (see Glossary) – glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) – orchestrate postprandial glucose and lipid metabolism [3]. This helped to determine how the consumption of macronutrients triggers a response from the gastrointestinal tract that promotes energy disposal to maintain glycemic control. It is now established that an integrated network of neuronal and endocrine signals achieves this by regulating gastric transit, stimulating insulin secretion, and activating satiety pathways that innervate the central nervous system (CNS) [4]. Incretins are among the gut-produced factors released upon meal ingestion to govern postprandial metabolism. Once secreted, GIP and GLP-1 activate their receptors on pancreatic β cells to enhance an insulinotropic response, that is both glucose-dependent and proportional, to drive disposal of the absorbed carbohydrate and lipid load [5]. Experimentally, this phenomenon is defined as the enhancement of insulin secretion in response to enteral versus intravenous glucose administration [6], that is known as the ‘incretin effect’.

In T2DM, the incretin effect is impaired, which may contribute to poor postprandial glycemic control [7]. Studies performed more than 25 years ago indicated that meal-stimulated concentrations of GLP-1 were reduced in T2DM, but the insulinotropic response upon infusion of pharmacological concentrations of GLP-1 was similar between diabetic and normoglycemic subjects, supporting the potential of GLP-1-based medicines [7,8]. Further infusion studies revealed a satiety effect of GLP-1, indicating a possible therapeutic benefit on energy consumption [9]. Coincident with advances in biotherapeutic engineering, these studies inspired drug discovery campaigns on GLP-1 over the subsequent two decades that delivered GLP-1 receptor agonists (GLP-1RAs) for treating T2DM. These became the first glucose-lowering medications to reduce body weight, and some have now been shown to provide cardiovascular health benefits [10].

Highlights
Novel ligands possessing agonist activity at both GIP and GLP-1 receptors are being investigated for the treatment of T2DM.

The GIP component of dual GIP/GLP-1 receptor agonism is hypothesized to act centrally to enhance GLP-1-induced weight loss.

The ability of GIP to target white adipose tissue (WAT) and improve its lipid buffering capacity is proposed to protect from ‘spillover’ of dietary lipids.

Pairing the anorexigenic effects of GIP/GLP-1 receptor agonism with the peripheral actions of GIP to promote lipid storage in WAT may be advantageous over the mechanisms of current treatments for T2DM.
Although GLP-1RAs are popular medicines today, the development of these agents also provided insights to consider when contemplating the therapeutic concepts of tomorrow. Among these, two important observations are that high and sustained concentrations of GLP-1RA lead to more robust glucose lowering and weight loss, and conversely that gastrointestinal effects such as nausea and vomiting limit reaching the maximal therapeutic potential of GLP-1RAs. Infusion studies originally demonstrated the advantage of high and constant levels of GLP-1 [11], and half-life extension strategies have shown the benefit of sustained agonism versus shorter-acting agents [12,13]. However, because gastrointestinal effects prevent aggressive dose escalation [14], titration schemes were necessary to slowly increase drug levels [15]. Even with these regimens, some patient attrition occurs that is attributed to gastrointestinal side effects.

Because the dose-tolerance for GLP-1R activation may impact on patients reaching glycemic and weight loss goals, additional therapies are being sought. Medicines providing the benefit of sustained GLP-1R activation while minimizing tolerability concerns and/or improving metabolic homeostasis are desired. One concept is to design multifunctional agents that possess GLP-1RA pharmacology as a foundation, but that also target alternative pathways to expand the therapeutic index [16–18]. A compelling approach may be to combine GLP-1 with an additional pharmacology that promotes the physiologic improvements fostered by moderate weight loss and enhanced white adipose tissue (WAT) function (Box 1), including improved lipid metabolism and systemic insulin sensitivity [19]. Therefore, given its beneficial effects on macronutrient metabolism and energy consumption, the partner incretin GIP is an attractive candidate to pair with the GLP-1RA mechanism.

Box 1: WAT Is a Key Buffer of Dietary TAG

WAT is a highly dynamic, multifunctional, and proficient storage site that is characterized by the presence of a heterogeneous population of cells, including adipocytes, preadipocytes, immune cells, endothelial cells, and fibroblasts, that together function to facilitate healthy expansion in response to caloric excess [96]. Adipocytes, the primary cell type in WAT, are equipped for lipid storage, breakdown, and hormonal (adipokine) secretion, and thereby play a key role in maintaining energy balance and systemic insulin sensitivity [37]. The largest sites of WAT in adult humans are the so-called ‘healthy’ subcutaneous WAT and ‘unhealthy’ visceral WAT deposits [98]. Subcutaneous WAT is located under the skin and accounts for the majority of triglyceride storage, and upper-body subcutaneous WAT is the primary supplier of systemic energy needs; visceral WAT is found within the abdominal wall, and its accumulation is a risk factor for developing metabolic disease [99].

Although WAT is now recognized as a key endocrine organ, its primary role is to function as a daily buffer for circulating lipids [29]. In the healthy lean state, during the postprandial period, WAT takes up and stores dietary lipid via the hydrolysis of circulating triglycerides by insulin-activated lipoprotein lipase (LPL), a lipase that is bound to the capillaries located on the endothelium of WAT. In the postabsorptive state (during fasting), stored triglycerides are broken down by intracellular lipases (HSL, ATGL) located on lipid droplets, and are released into the circulation as free fatty acids for use as an energy source by metabolically active tissues. The flexibility of WAT to switch between energy storage and delivery in the fed and fasted states, respectively, is indicative of its metabolic health. This has been shown by numerous studies to be fundamental to maintaining systemic metabolic function [90]. To ensure its buffering capability in response to a positive energy balance, WAT has an immense capacity to remodel and expand its storage ability, thus accommodating the accumulation of surplus energy while maintaining functionality. The expansion of WAT occurs by two mechanisms: adipocyte hypertrophy (increased cell size) and adipocyte hyperplasia (increased cell number). Hyperplasia is considered to be the primary mechanism by which ‘healthy’ adipose tissue expansion occurs [49], a process influenced by the ability of the surrounding extracellular matrix to promote remodeling as well as to enhance the generation of new blood vessels.

However, in the context of prolonged positive energy balance and obesity, the expansion capacity of WAT becomes rate-limiting [100]. If the storage capacity of WAT is exceeded, enlarged and stressed adipocytes become hypoxic, and proinflammatory immune cells infiltrate the tissue, resulting in fibrosis and adipocyte death. Once this happens, excess calories can no longer be stored properly, and dietary lipids are taken up by the liver, skeletal muscle, and pancreas [21]. If not corrected, ectopic lipid accumulation occurs, causing lipotoxicity that promotes insulin resistance, often leading to the development of T2DM [21].
GIP Enhances the Lipid-Buffering Capacity of WAT

Although the insulinotropic actions of GIP would be expected to improve glycemic control, studies performed since its discovery (Figure 1) indicate that an equally important role of GIP may be to promote storage of dietary lipid. Unlike GLP-1R, the GIP receptor (GIPR) is expressed in WAT [20], and GIPR agonism is hypothesized to enhance the ability of adipocytes to acutely clear dietary triglyceride (TAG), as well as improve the long-term storage of lipids through facilitating the healthy expansion of WAT (Box 1). Expanding WAT to store calories reduces lipid ‘spillover’ and ectopic fat accumulation in tissues such as liver, skeletal muscle, heart, and pancreas [21].

The primary physiological role of WAT is to function as a daily buffer of circulating lipids [22], releasing free fatty acids (FFAs) in the fasted state and storing dietary lipid in the fed state (Box 1). In T2DM, the lipid-buffering capacity of WAT is dysregulated because storage capacity is exceeded and is accompanied by reduced insulin-mediated suppression of FFA release, lowered adipose tissue perfusion (blood flow), and impaired recruitment of lipoprotein lipase (LPL) [23–28]. In dog and rodent models, GIP can promote lipid disposal, as demonstrated by its ability to enhance TAG clearance, lower TAG excursions following an intraduodenal lipid load, and increase LPL activity and TAG synthesis in WAT [29–31]. Further, inhibition of GIP following oral lipid administration increases postprandial TAG levels [32–34]. In humans under experimental conditions simulating the postprandial period,
GIP increases WAT blood flow, lowers FFAs, and stimulates glucose uptake and TAG storage in WAT [35]. Importantly, GIP infusion can drive TAG storage in WAT and reduce circulating FFAs in T2DM patients [36], although there is evidence that the lipogenic effect of GIP on WAT blood flow and TAG deposition is attenuated in T2DM [37], an effect possibly linked to reduced GIPR expression in WAT [36,38]. However, in follow-up experiments, the ability of GIP to increase WAT perfusion and TAG storage was rescued by weight loss [39].

GIP promotes TAG storage following food intake by directly activating GIPR on adipocytes, indirectly through the lipogenic actions of insulin, or through the combination of the two [40,41]. The GIPR is expressed in both visceral and subcutaneous WAT [20,32,38] and has been shown to be functionally active in many species, including rodent and human adipocyte systems [42–44]. In the absence of insulin, GIP increases LPL activity (Box 1) in both pre- and mature 3T3-L1 adipocytes [30,45]. Further, GIP is a potent insulin-sensitizer in adipocytes [46,47] and is capable of enhancing glucose uptake through glucose transporter type 4 (GLUT4) translocation and chylomicron TAG hydrolysis through LPL activity [30,48]. These effects do not appear to occur through classical GIPR signaling, but instead through the PI3K/PKB (phosphoinositide-3-kinase/protein kinase B) pathway, leading to GLUT4-mediated glucose uptake and via PI3K/PKB-regulated inhibition of LKB1/AMPK activity, pCREB recruitment, and the nuclear translocation and transcriptional activity of target of rapamycin complex 2 (TORC2) for LPL recruitment [30,47,48]. Together, GIP can target WAT to increase its perfusion (ensuring optimal nutrient and oxygen delivery), recruit LPL (maximizing the hydrolysis of TAG and release of FFA), enhance insulin-stimulated glucose uptake (required for generating glycerol-3-phosphate), and subsequently drive lipid uptake and storage (Figure 2).

GIP may also improve energy storage by facilitating WAT expansion (Box 1). This can occur by hypertrophy of existing adipocytes and/or by differentiation of preadipocytes, namely de novo adipogenesis, where increasing adipocyte numbers is the primary way in which healthy expansion occurs [49]. The GIPR is found at low levels in preadipocytes, but its expression increases throughout differentiation and is associated with markers of adipocyte development [50,51]. PPARE (peroxisome proliferator-activated receptor γ) is a master regulator of WAT generation, GIPR is a downstream PPARE target, and PPARE ligands increase its expression whereas PPARE knockdown decreases GIPR levels [52,53]. Similarly, GIP enhances adipocyte

Box 2. CNS Regulation of Energy Balance

Energy balance is controlled by regions of the CNS such as the hypothalamus and caudal brainstem [101]. The hypothalamus is recognized as the ‘command center’ for energy balance regulation and is composed of discrete nuclei that are fundamental for energy homeostasis, including the arcuate nucleus (ARC), paraventricular nucleus, dorsomedial nucleus, ventromedial nucleus, and lateral hypothalamus [102]. The ARC is located adjacent to the third ventricle and median eminence where the blood-brain barrier is incomplete, allowing direct sensing of both short-term (e.g., glucose and FFA) and long-term (e.g., insulin and leptin) signaling of energy status [103]. The ARC contains two major neuronal populations that control caloric intake and expenditure [104]. These first-order neurons express the orexigenic neuropeptides neuropeptide Y and agouti-related peptide that drive food intake or anorexigenic neuropeptides derived from pro-opiomelanocortin and from cocaine and amphetamine-regulated transcript that reduce caloric intake [104]. In addition to the hypothalamus, the brainstem plays an important role in the control of food intake by responding to short-term satiety signals [105]. Within the brainstem, the dorsal vagal complex, that includes the area postrema (AP), nucleus of the tractus solitarius (NTS), and dorsal motor nucleus of the vagus, controls meal size and frequency. Similarly to the hypothalamus, these nuclei are located in close proximity to the ventricular system (i.e., the fourth ventricle) and thus have direct access to circulating hormones and nutrients [57,105]. Through the AP and NTS, the brain sense, monitors, and integrates dietary nutrient (glucose), hormonal (AP-mediated direct sensing of gut peptides GLP-1, PYY, etc.), and neural (gut peptide-induced vagal afferent signaling to the NTS) signals from the gastrointestinal tract [57]. This ‘peripheral’ information is relayed to higher brain centers such as the hypothalamus to reduce food intake. Finally, caloric intake and therefore energy balance is also modulated by brain regions associated with motivational and reward-related feeding, including the ventral tegmental area, nucleus accumbens, and amygdala that, together with the drive to feed, ensure the attainment and consumption of highly palatable and energy-dense food [57].
differentiation [50], and knockdown of GIPR impairs adipocyte development [51]. In total, these findings support the potential of GIPR agonism to ensure the lipid-buffering capacity of WAT, reducing lipid ‘spillover’ in the face of a positive energy balance (Figure 2). Future investigations will be necessary to fully test this hypothesis.

The importance of the WAT effects of GIP is exemplified by pharmacological and genetic studies showing that continued engagement of GIPR in models of insulin resistance improves whole-body insulin sensitivity and lowers hepatic lipid accumulation [54,55]. These effects are associated with improved WAT health, indicated by lowered proinflammatory immune cell infiltration/cytokine secretion, the induction of genes associated with enhanced adipocyte lipid storage capacity and TAG storage, and the release of insulin-sensitizing adipokines [54,55]. Although some of the metabolic improvement may be due to the incretin action of GIP, the benefits related to WAT function and insulin sensitivity support a direct action of GIP pharmacology on adipocytes (Figure 2).

GIP Can Act in the CNS to Lower Food Intake and Reduce Body Weight

In addition to improving lipid handling in the periphery, GIP action in the CNS may provide further metabolic benefits by reducing energy consumption, especially when combined with GLP-1. This has been demonstrated in high-fat fed mice where administration of both GIP and GLP-1 caused more robust anorexia and weight-lowering than the individual agents [56]. Although the effects of GLP-1 in areas of the CNS associated with homeostatic and reward related feeding have been established [57], the data on the combination of the two agents prompt questions about the potential central action of GIP (see Outstanding Questions).

Several lines of evidence support a central mode of action for GIP (Figure 1). The GIPR is widely expressed within the CNS [58,59] and is found in areas implicated in regulating energy balance [60,61]. Although the data are limited, GIP appears to be capable of crossing the blood–brain barrier to access sites of action [58]. For metabolic disease, experiments show that GIPR agonism can drive weight loss. For example, chronically elevated GIP in transgenic mice reduces diet-induced obesity (DIO) and improves insulin sensitivity by reducing caloric intake [54]. In this model, GIP is highly expressed in the hypothalamic ventromedial nucleus, hinting at direct central action [54]. In support, the GIPR is found in the hypothalamus of adult humans and mice [61]. In studies using GIPR reporter mice, Adriaenssens et al. reported that GIPR is expressed in arcuate (ARC), paraventricular, and dorsomedial nuclei, hypothalamic centers that are associated with energy balance (Box 2) [61]. A key finding of this work is that, within the hypothalamus, GIPR is expressed by cells lacking GLP-1R but also in cells where GLP-1R is found (in mice and humans) [61]. Therefore, GIP/GLP-1 synergism may occur by activating each receptor on separate cells (classical signaling pathways), on the same cell (generating a unique signal), or the downstream integration of both. Other studies indicate that GIP and GLP-1 activate distinct neurons when inhibiting food intake [62]. Notably, GIPR is expressed in non-neuronal cells of the mediobasal hypothalamus [61], including oligodendrocytes that regulate the access of peripheral signals to the ARC [63]. This raises the possibility that GIP may enhance GLP-1 function by increasing its access to anorexigenic neuronal populations within the mediobasal hypothalamus. Intriguingly, GIPR-expressing cells within the ARC have receptors for neuropeptides that are linked to appetite control; these neurons respond to ligands that regulate feeding. Accordingly, chemogenetic activation of GIPR-positive cells reduces caloric intake [61]. Further, other studies show that central and peripheral administration of GIPR agonists lowers body weight by reducing caloric intake [62,64], effects that may occur by GIP recruiting neuropeptides linked to appetite control [65]. In addition to the ARC, the brainstem controls food intake by responding to gut-derived satiety factors (Box 2). GIPR has been shown to be expressed by brainstem nuclei [61]; however, it is not known if GIP can act in this region of the CNS to affect feeding behavior.
A factor affecting the tolerability of GLP-1RA treatment is the occurrence of drug-related nausea [14]. Interestingly, GIPR agonism attenuates the aversive and emetic responses characteristic of the gut peptide PYY and the oncology drug cisplatin, respectively [66]. This suggests a novel hypothesis where GIP may enhance GLP-1R-mediated weight loss by increasing tolerance, thereby expanding the therapeutic index. Together, current evidence suggests that GIP has the potential to drive weight loss by directly targeting its receptor in the CNS to inhibit caloric intake, by enhancing the anorectic action of GLP-1, or by reducing drug-induced nausea to expand GLP-1RA efficacy, or combinations thereof (Figure 2).

The GIP Conundrum
Despite the appealing benefits of GIP, its therapeutic potential is controversial. This is in part because of early findings that GIP resistance manifests in the diabetic condition, but also because of concerns that the lipogenic effects of GIP could promote weight gain. Although some of the complexity is related to how significant GIP resistance is to the impaired incretin effect in T2DM, the role of GIP in non-pancreatic tissues to regulate energy metabolism continues to be debated. For insulin secretion, by contrast to GLP-1, the early infusion studies showed there is a poor insulinotropic response to GIP in T2DM patients, although these studies employed physiologic rather than pharmacologic concentrations of GIP [8,67]. These findings hampered initial therapeutic ambitions for GIPR agonists. However, interest in agonists was rekindled when subsequent studies showed that GIP sensitivity in T2DM can be restored by improving glucose control [68]. Recently, new studies suggest that GIP is the predominant incretin [69], highlighting the importance of GIP in insulin secretion. However, it should also be noted that the incretins appear to have opposing effects on the counter-regulatory hormone glucagon, because GLP-1 decreases [70], whereas GIP increases its secretion [71]. However, the glucagon response to GIP is not fully understood because GIP stimulates glucagon in hyperglycemia in T2DM patients [72], but not in healthy subjects – where an increase only occurs in hypoglycemic conditions [73]. From a therapeutic standpoint, the consequence of GIP-stimulated glucagon secretion on glucose control, and possibly on energy expenditure, requires further investigation [74]. Irrespective, in T2DM patients, acute coadministration of GIP and GLP-1 has a neutral effect on glucagon [72].

Although the insulinotropic activity of GIP is now clarified, the hypothesis that GIP blockade lowers body weight persists. This was postulated upon discovering that Gipr loss of function prevents DIO, and was further substantiated by studies showing that crossing Gipr null animals with ob/ob mice reduces adiposity [75], findings subsequently bolstered by adipose tissue-specific rescue and knockout of Gipr in mouse models [76,77]. The observation that Gipr null mice are resistant to weight gain aligned with the obesogenic view of GIP, and together inspired the pursuit of GIPR antagonists. However, adipose tissue-specific deletion and rescue Gipr models used the aP2 promoter, which is known to be expressed in multiple cell types (macrophages, neurons [78]), potentially clouding the interpretation of these studies. Furthermore, for germline whole-body Gipr null mice, an alternative explanation for the protection from obesity could be that metabolic rate is increased due to thermal stress. Gipr knockout animals have elevated expression of the thermogenic protein, uncoupling protein 1, in brown adipose tissue (BAT), and these mice show an enhanced ability to sustain body temperature in response to cold exposure [32]. Further, BAT-specific Gipr null mice have a similar phenotype, but if maintained in thermal neutrality the enhanced thermogenic capacity attenuates [32]. Thus, protection from DIO in these models appears to be largely due to cold intolerance when housed at ambient temperatures, similar to findings of other genetic models [79].

Other studies have also resulted in conflicting conclusions regarding GIPR agonism versus antagonism. For example, both immunization against GIP [80] and immunoneutralization of GIP [81]
reduce body weight gain in mice. Similarly, recently developed GIPR antagonist antibodies blunt weight gain in preclinical models [82], although this pharmacology is not observed with GIPR antagonist peptides [64]. The antagonist antibody data are by contrast to conclusions drawn from GIP transgenic mice that are resistant to DIO [54] and from the weight-reducing effects of newly developed GIPR agonists [64] (discussed earlier). To reconcile the agonist versus antagonist discrepancy, it has been proposed that chronic agonism may cause GIPR desensitization, thereby diminishing GIP activity [82,83], to date, supporting evidence has not been reported. Further, there is no evidence that GIPR blockade reduces hyperglycemia. Although body weight improvements are observed with both modalities, the most beneficial metabolic effect of targeting the GIP system appears to occur when adding a GIPR agonist to GLP-1RA therapy [56].

The Dual GIP/GLP-1 Hypothesis
Activating both the GIP and GLP-1 receptors is especially attractive in the treatment of T2DM because the combined mechanisms may enhance insulin secretion, decrease energy consumption, and both directly and indirectly improve insulin sensitivity (Figure 2). The approach is feasible because improved glycemia restores sensitivity to GIP [68], and peptide engineering enables the design of hybrid ligands that exhibit dual agonism [84,85]. Strong effects of dual agonism are predicted for insulin secretion because both receptors are found in pancreatic β cells; however, the CNS shows both overlapping and differential receptor distributions in various metabolic centers, and adipocytes only express GIPR. Activation of GIPR in the brain and WAT could be uniquely complementary to GLP-1R signaling. If GIP expands the tolerance window of GLP-1R function, targeting both systems may offset and augment the effects of the individual modalities.

Figure 2. Schematic Depiction of the Pleiotropic Benefits of Dual Glucose-Dependent Insulinotropic Polypeptide (GIP)/Glucagon-Like Peptide-1 (GLP-1) Receptor Agonist Therapy in Type 2 Diabetes Mellitus. Activating both the GIP and GLP-1 receptors is attractive because the combination of these mechanisms is hypothesized to enhance glucose-dependent insulin secretion, decrease energy consumption, and both directly and indirectly improve white adipose tissue health and function and subsequently whole-body insulin sensitivity. This figure was created using BioRender (https://biorender.com).
1RAs, and GIP improves WAT function and lipid handling, dual incretin agonists may advance therapeutic outcomes.

**Clinical Signs of a GIP Benefit**

Finan *et al.* investigated the dual GIP/GLP-1 receptor agonist hypothesis by engineering the glucagon core sequence to yield a peptide that has balanced agonism at each receptor [56]. Subsequently, this molecule was conjugated either to a C16 saturated fatty acyl chain for once-daily dosing or to polyethylene glycol (PEG) to support once-weekly dosing. Preclinical dose response studies in insulin-resistant and frankly diabetic rodents established that the acylated peptide exhibits superior glucose and body weight lowering efficacy compared with a selective GLP-1RA. Importantly, the C16 conjugated peptide liraglutide was employed as the GLP-1RA comparator in these studies because it is well matched to the dual agonist in terms of pharmacokinetic profile and GLP-1R potency. In a 6 week study in T2DM subjects, the PEGylated GIP/GLP-1 agonist dose-dependently lowered HbA1c from baseline, with a maximal decrease of 1.11% at the highest dose (30 mg) [56]. However, in the absence of a GLP-1 RA comparator, it was not possible to reach conclusions about the relative glucose-lowering efficacy of the dual agonist. Body weight data were not reported for this study, and no further clinical evaluation of the PEGylated dual agonist has been reported. More substantial investment has been made in evaluating the clinical efficacy of the acylated version of this GIP/GLP-1 agonist [86–88]. This molecule has variously been known as MAR709, RG7697, and NNC00902746, reflecting its successive ownership by three biopharmaceutical companies [89]. In a 12 week trial in T2DM patients, this molecule improved glycemic control (HbA1C reduction of 1.5%) and reduced body weight (3.5%) at the single daily dose evaluated of 1.8 mg. Liraglutide was included in this study as an open-label arm to relate to historical studies, but the authors cautioned against its use as an active comparator [88]. Post hoc analysis of the study revealed that NNC00902746 caused significantly greater weight loss in patients with baseline HbA1c <8.5%, and a similar but a nonsignificant trend was observed in change of HbA1c. The latter counters what is usually reported in the study of glucose-lowering drugs but supports the notion that GIP incretin pharmacology may become more evident upon restoration of pancreatic β cell GIP sensitivity as glucose control improves [68]. Suggestive of an insulin-sensitizing effect, NNC00902746 improved glucose tolerance, accompanied by reduced insulin secretion, at doses where decreased body weight was not observed in either diabetic rats [56] or T2DM patients [87]. Might these data be evidence in support of GIPR agonism contributing to the efficacy of NNC00902746?

Unfortunately, no further clinical development of this agent has been reported that could corroborate the dual GIP/GLP-1 receptor agonist concept for this molecule. Tirzepatide (LY3298176) is a dual GIP/GLP-1 receptor agonist engineered from the GIP sequence that has GIPR activity equal to that of native GIP but is somewhat less potent than GLP-1 at the GLP-1R [90]. The properties of tirzepatide, including modification with a C20 unsaturated di-acid acyl chain, allow once-weekly dosing [90]. In a 26 week trial in T2DM patients (Phase IIb), tirzepatide demonstrated superior glucose control and body weight lowering compared with the GLP-1RA, dulaglutide [91]. Tirzepatide reduced HbA1C by 1.6%, 2.0%, and 2.4% in the 5 mg, 10 mg, and 15 mg dose groups, respectively, compared with 1.1% for dulaglutide 1.5 mg. Importantly, 18% of patients receiving 10 mg and 30% dosed with 15 mg reached normoglycemia (HbA1C <5.7%) compared with 2% of subjects administered dulaglutide [91]. Further, more patients treated with 5 mg, 10 mg, and 15 mg reached body weight targets (>5%, >10%, and >15% weight loss from baseline) versus those administered dulaglutide. Although appetite was not directly assessed, adverse-event reporting indicated that decreased appetite was more common with tirzepatide treatment than with dulaglutide. For the dual agonist concept, this is in line with the putative role of GIP to complement the anorectic action of GLP-1 in the CNS, as suggested by preclinical pharmacology. The incidences of gastrointestinal effects (nausea, diarrhea, and vomiting)
were similar for tirzepatide at 5 mg (26%) and 10 mg (39%) with dulaglutide (35%), but higher at the 15 mg dose (60%). Hence, tirzepatide at 5 mg and 10 mg provided superior glycemic and body weight control versus dulaglutide, but with similar tolerability. This indicates that the GIP/GLP-1 mechanism achieves greater efficacy in part by allowing dose escalation above GLP-1RAs. Recent data suggest that lower starting doses and smaller dose increments provide a more favorable side effect profile [92], offering the prospect that more patients could benefit from the high dose of tirzepatide.

Insulin sensitivity, assessed indirectly by fasting biomarkers (glucose and insulin) and homeostasis model assessment-2 insulin resistance (HOMA2-IR), was improved by tirzepatide. Post hoc regression analysis of HOMA2-IR suggests that only 20–30% of the improvement in insulin sensitization by tirzepatide is due to weight loss [93], by contrast to GLP-1RAs where insulin sensitivity is attributed to weight loss only [94]. Additional studies with tirzepatide will more directly explore the effect of GIP/GLP-1 receptor agonism on pancreatic α cell function, β cell function, and whole-body insulin sensitivity (ClinicalTrials.gov identifier NCT03951753).

In light of the potential benefit of GIPR agonism on peripheral insulin sensitization, the GIP component of dual agonism may account for the added benefit. The mechanistic basis for weight-independent insulin sensitization merits further research to assess the direct effects of GIP on WAT and lipid metabolism. Accordingly, tirzepatide more effectively lowered fasting TAGs than did dulaglutide [81]. In addition, tirzepatide lowered ApoB-containing lipoproteins and atherogenic lipoprotein subclasses. For example, fasting levels of larger TAG-rich particles and small dense LDL particles were reduced [95]. Hence, the dual agonist mechanism appears to improve the atherogenic lipoprotein profile.

**Concluding Remarks**

The tirzepatide Phase IIb data establish clinical proof of concept that combining GIP and GLP-1 receptor agonism leads to superior efficacy. The strong glucose lowering of dual agonism is consistent with enhanced insulinotropic action and the weight-independent insulin-sensitizing effects of GIP. Robust weight loss is in line with the proposed synergy of the incretins in the CNS to promote satiety, and the apparent weight-independent effects of GIP/GLP-1 receptor agonism are likely the result of better lipid handling fostered by improved WAT health.

The Phase III program for tirzepatide will inform on the longer-term efficacy and safety of dual GIP/GLP-1 receptor agonism in both T2DM and obesity. Of particular interest will be outcomes of the SURPASS-2 trial where tirzepatide is studied versus the GLP-1RA semaglutide (ClinicalTrials.gov identifier NCT03987919). Much remains to be learned regarding the molecular mechanism of action that underpins the efficacy of dual GIP/GLP-1 receptor agonism (see Outstanding Questions). Progress will be hard fought and will require concerted research both preclinically and in man. More tools (pharmacological and genetic) are available for use in rodents, but preclinical findings must ultimately be validated in man to confirm their translational relevance. The 2020s herald ever more intense research into the mechanism of action of GIP/GLP-1 receptor agonism.

**Acknowledgments**

We thank Minrong Ai, Omer Cabrera, Michael E Christe, Tamer Coskun, Paul Emmerson, Laura Fernandez, Robert Heine, Chrisanthi Karanikas, Clare Lee, Julie Moyers, Paul Owens, James Perfield, William Roell, Shweta Urva, and Ruth Gimeno for helpful discussions on the topics of this manuscript.
References

1. Best, C.H. (1945) Insulin and diabetes – in retrospect and in prospect: the Banting Memorial Lecture, 1945. Can. Med. Assoc. J. 53, 204–212
2. Unger, R.H. (1971) Glucagon physiology and pathophysiology. N. Engl. J. Med. 285, 443–449
3. Holst, J.J. (1994) Glucagon-like peptide 1: a newly discovered gastrointestinal hormone. Gastroenterology 107, 1848–1855
4. Kim, K.S. et al. (2018) Signaling from the periphery to the brain that regulates energy homeostasis. Nat. Rev. Neurosci. 19, 195–214
5. Baggio, L.L. and Drucker, D.J. (2007) Biology of incretins: GLP-1 and GIP. Gastroenterology 132, 2131–2157
6. Nauck, M.A. et al. (1986) Incretin effects of increasing glucose load in man calculated from venous insulin and C-peptide responses. J. Clin. Endocrinol. Metab. 63, 492–498
7. Nauck, M. et al. (1986) Reduced incretin effect in type 2 (non-insulin-dependent) diabetes. Diabetologia 29, 46–52
8. Nauck, M.A. et al. (1993) 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
9. Flier, J. et al. (1999) Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. J. Clin. Invest. 101, 515–520
10. Causso, I. et al. (2019) Heterogeneity and similarities in GLP-1 receptor agonist cardiovascular outcomes trials. Trends Endocrinol. Metab. 30, 578–589
11. Larsen, J. et al. (2001) Glucagon-like peptide 1 infusion must be maintained for 24 h/day to obtain acceptable glycemia in type 2 diabetic patients who are poorly controlled on sulphonylurea treatment. Diabetes Care 24, 1416–1421
12. Blevins, T. et al. (2011) DURATION-5: exendin once weekly resulted in greater improvements in glycemic control compared with exendin twice daily in patients with type 2 diabetes. J. Clin. Endocrinol. Metab. 96, 1301–1310
13. Wysham, C. et al. (2014) Efficacy and safety of dulaglutide added onto pioglitazone and metformin versus exenatide in type 2 diabetes in a randomized controlled trial (AWARD-1). Diabetes Care 37, 2159
14. Andersen, A. et al. (2018) Glucagon-like peptide 1 in health and disease. Nat. Rev. Endocrinol. 14, 390–403
15. Leen, M.D. et al. (2014) Tolerance of nausea and vomiting and associations with weight loss in a randomized trial of liraglutide. J. Clin. Endocrinol. Metab. 96, 1301–1310
16. Tschop, M.H. et al. (2016) Unimolecular polypharmacology for the treatment of diabetes and obesity. Cell Metab. 24, 51–62
17. Tschop, M. and DiMarchi, R. (2017) Single-molecule combinatorial therapeutics for treating obesity and diabetes. Diabetes 66, 1766–1769
18. Willard, F.S. et al. (2018) Beyond glucagon-like peptide-1: is G protein coupled receptor pharmacology the path forward to treating metabolic diseases? ACS Pharmacol. Transl. Sci. 1, 3–11
19. Wing, R.R. et al. (2011) Benefits of modest weight loss in improving cardiovascular risk factors in overweight and obese individuals with type 2 diabetes. Diabetes Care 34, 1481–1489
20. Rudovich, N. et al. (2007) GIP receptor mRNA expression in different fat tissue depots in postmenopausal non-diabetic and diabetic women. J. Endocrinol. 194, 153–159
21. DeFronzo, R.A. (2010) Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links. The Claude Bernard Lecture 2009. Diabetologia 53, 1270–1287
22. Farnsworth, K. (2000) Adipose tissue as a buffer for daily lipid flux. Diabetologia 43, 1201–1210
23. Cng, J.M. and Kern, P.A. (1989) Effect of feeding and obesity on lipoprotein lipase activity, insulinresistant, tissue, and messenger RNA levels in human adipose tissue. J. Clin. Invest. 94, 305–311
24. McQuaid, S.E. et al. (2011) Downregulation of adipose tissue fatty acid trafficking in obesity: a driver for ectopic fat deposition? Diabetes 60, 47–50
25. Lotta, L.A. et al. (2017) Integrative genomic analysis implicates limited peripheral adipose storage capacity in the pathogenesis of human insulin resistance. Nat. Genet. 49, 17–26
26. Campbell, P.J. et al. (1994) Fat metabolism in human obesity. Am. J. Physiol. 266, E603–E605
27. Karpe, F. et al. (2002) Effects of insulin on adipose tissue blood flow in man. J. Physiol. 540, 1087–1093
28. Karpe, F. et al. (2002) Impaired postprandial adipose tissue blood flow response is related to aspects of insulin sensitivity. Diabetes 51, 2467–2473
29. Oben, J. et al. (1991) Effect of the entero-pancreatic hormones, gastric inhibitory polypeptide and glucagon-like polypeptide-1[7–36] amide, on fatty acid synthesis in explants of rat adipose tissue. J. Endocrinol. 130, 267–272
30. Kim, S.J. et al. (2007) Activation of lipoprotein lipase by glucose-dependent insulinoitropic polypeptide in adipocytes. A role for a protein kinase B, LKB1, and AMP-activated protein kinase cascade. J. Biol. Chem. 282, 8557–8567
31. Wasada, T. et al. (1981) Effect of gastric inhibitory polypeptide on plasma levels of chylomicron triglycerides in dogs. J. Clin. Invest. 69, 1106–1107
32. Beaudry, J.L. et al. (2019) Physiological roles of the GIP receptor in murine brown adipose tissue. Mol. Metab. 29, 14–25
33. Aasman, M. et al. (2017) The gluco- and liporegulatory and vasodilatory effects of glucose-dependent insulinoitropic polypeptide (GIP) are abolished by an antagonist of the human GIP receptor. Diabetes 66, 2363–2371
34. Elbert, R. et al. (1991) Effect of exogenous or endogenous gastric inhibitory polypeptide (GIP) on plasma triglyceride responses in rats. Horm. Metab. Res. 23, 517–521
35. Aasman, M. et al. (2010) Glucose-dependent insulinoitropic polypeptide may enhance fatty acid re-esterification in subcutaneous abdominal adipose tissue in lean humans. Diabetes 59, 2160–2163
36. Thondam, S.K. et al. (2017) Glucose-dependent insulinoitropic polypeptide promotes lipid deposition in subcutaneous adipocytes in obese type 2 diabetes patients: a maladaptive response. Am. J. Physiol. Endocrinol. Metab. 312, E224–E223
37. Aasman, M. et al. (2014) Glucose-dependent insulinoitropic polypeptide has impaired effect on abdominal, subcutaneous adipose tissue metabolism in obese subjects. Int. J. Obes. 38, 259–265
38. Ceperuelo-Balleste, V. et al. (2014) Disruption of GIP/GPR119 acts in human adipose tissue is linked to obesity and insulin resistance. J. Clin. Endocrinol. Metab. 99, E909–E919
39. Aasman, M. et al. (2016) The blunted effect of glucose-dependent insulinoitropic polypeptide in subcutaneous abdominal adipose tissue in obese subjects is partly reversed by weight loss. Nutr. Diabetes 6, e209
40. Aasman, M. et al. (2016) Insulin plays a permissive role for the vasoadective effect of gip regulating adipose tissue metabolism in humans. J. Clin. Endocrinol. Metab. 101, 3155–3162
41. Campbell, J.E. et al. (2016) TCF1 links GIP signaling to the control of beta cell function and survival. Nat. Med. 22, 84–90
42. Beck, B. and Max, J.P. (1983) Gastric inhibitory polypeptide enhancement of the insulin effect on fatty acid incorporation into adipose tissue in the rat. Regul. Pept. 7, 3–6
43. Yip, R.G. et al. (1998) Functional GIP receptors are present on adipocytes. Endocrinology 139, 4004–4007
44. Kim, S.J. et al. (2013) GIP increases human adipocyte LPL expression through PPARδ and TORC2-mediated trans-activation of the LPL gene. J. Lipid Res. 51, 3145–3157
45. Eckel, R.H. et al. (1979) Gastric inhibitory polypeptide enhanced lipoprotein lipase activity in cultured preadipocytes. Diabetes 28, 1141–1142
46. Stark, G.H. et al. (1985) GIP increases insulin receptor affinity and cellular sensitivity in adipocytes. Am. J. Physiol. 249, E603–E607
47. Mohammad, B. et al. (2011) Gastric inhibitory peptide controls adipose insulin sensitivity via activation of cAMP-response element-binding protein and p110beta isoform of phosphatidylinositol 3-kinase. J. Biol. Chem. 286, 43062–43070
48. Mohammad, B. et al. (2014) A naturally occurring GIP receptor variant underlies enhanced agonist-induced desensitization, which impairs GIP control of adipose insulin sensitivity. Mol. Cell. Biol. 34, 3618–3629
insulin sensitivity in type 2 diabetes patients. Diabetologia 62, S1–S600

94. Fonseca, V.A. et al. (2019) Reductions in insulin resistance are mediated primarily via weight loss in subjects with type 2 diabetes on semaglutide. J. Clin. Endocrinol. Metab. 104, 4078–4086

95. Wilson, J.E. et al. (2019) The dual GIP/GLP-1 receptor agonist tirzepatide improves lipoprotein biomarkers associated with insulin resistance and cardiovascular risk in patients with type 2 diabetes. Diabetologia 62, S1–S600

96. Crewe, C. et al. (2017) The ominous triad of adipose tissue dysfunction: inflammation, fibrosis, and impaired angiogenesis. J. Clin. Invest. 127, 74–82

97. Funke, J.B. and Scherer, P.E. (2019) Beyond adiponectin and leptin: adipose tissue-derived mediators of inter-organ communication. J. Lipid Res. 60, 1648–1684

98. Geossenn, G.H. (2017) The metabolic phenotype in obesity: fat mass, body fat distribution, and adipose tissue function. Obes. Facts 10, 207–215

99. Frayn, K.N. (2018) Turning over our fat stores: the key to metabolic health. Blaxter Award Lecture 2018. Proc. Nutr. Soc. 78, 398–406

100. Gray, S.L. and Vidal-Puig, A.J. (2007) Adipose tissue expandability in the maintenance of metabolic homeostasis. Nutr. Rev. 65, S7–S12

101. Yeo, G.S. and HeUSER, L.K. (2012) Unraveling the brain regulation of appetite: lessons from genetics. Nat. Neurosci. 15, 1343–1349

102. Gao, Q. and Horvath, T.L. (2007) Neurobiology of feeding and energy expenditure. Annu. Rev. Neurosci. 30, 367–398

103. Rodriguez, E.M. et al. (2005) Hypothalamic tanycytes: a key component of brain-endocrine interaction. Int. Rev. Cytol. 247, 89–164

104. Cone, R.D. (2005) Anatomy and regulation of the central melanocortin system. Nat. Neurosci. 8, 571–578

105. Grill, H.J. and Hayes, M.R. (2012) Hindbrain neurons as an essential hub in the neuroanatomically distributed control of energy balance. Cell Metab. 16, 296–309

106. Brown, J.C. and Dryburgh, J.R. (1971) A gastric inhibitory polypeptide II. The complete amino acid sequence. Can. J. Biochem. 49, 867–872

107. Dupre, J. et al. (1973) Stimulation of insulin secretion by gastric inhibitory polypeptide in man. J. Clin. Endocrinol. Metab. 37, 826–828

108. Nörnlund, H. et al. (1981) Amino acid sequence and heterogeneity of gastric inhibitory polypeptide (GIP). FEBS Lett. 123, 205–210

109. Mentlein, R. et al. (1993) 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

110. Gremlich, S. (1995) Cloning, functional expression, and chromosomal localization of the human pancreatic islet glucose-dependent insulinotropic polypeptide receptor. Diabetes 44, 1002–1006

111. Irwin, N. et al. (2007) Comparison of the anti-diabetic effects of GIP- and GLP-1-receptor activation in obese diabetic (Ob/Ob) mice: studies with DPP IV resistant N-AcGIP and exendin(1-39)amide. Diabetes Metab. Res. Rev. 23, 572–579