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Review

Transforming obesity: The advancement of multi-receptor drugs

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SUMMARY

For more than a century, physicians have searched for ways to pharmacologically reduce excess body fat. The tide has finally turned with recent advances in biochemically engineered agonists for the receptor of glucagon-like peptide-1 (GLP-1) and their use in GLP-1-based polyagonists. These polyagonists reduce body weight through complementary pharmacology by incorporating the receptors for glucagon and/or the glucose-dependent insulinotropic polypeptide (GIP). In their most advanced forms, gut-hormone polyagonists achieve an unprecedented weight reduction of up to ~20%–30%, offering a pharmacological alternative to bariatric surgery. Along with favorable effects on glycemia, fatty liver, and kidney disease, they also offer beneficial effects on the cardiovascular system and adipose tissue. These new interventions, therefore, hold great promise for the future of anti-obesity medications.

INTRODUCTION

Obesity, characterized by excessive body fat, constitutes a major risk factor for the development of type 2 diabetes (T2D), dyslipidemia, cardiometabolic disease,[1] cancer,[2] and overall mortality.[3] It further enhances complications associated with infectious diseases, as exemplified by coronavirus disease 2019 (COVID-19).[4] Between 1975 and 2014, global obesity rates escalated from 105 to 641 million adults (4% to 13% of the total population, respectively).[5] It is estimated that worldwide obesity will continue to rise to one billion adults by 2030,[6] irrespective of gender, geography, or rural and urban styles of living.[7] Lifestyle modifications, with increased physical exercise and/or reduced caloric intake, are hallmarks of any successful weight loss intervention.[8] However, despite appreciable weight loss of ~5%–8% in the short term, such lifestyle interventions have only limited potential for sustained weight reduction, particularly when utilized as a stand-alone therapy.[8] This is exemplified by a recent meta-analysis showing that ~56% of body weight loss, as achieved through lifestyle intervention, is regained within 2 years, and ~79% is regained after 5 years.[9] The major challenge in obesity management is the system's intrinsic drive to preserve energy to defend the higher body weight. A reduction in caloric intake is therefore often accompanied by a decrease in energy expenditure, along with enhanced sensitivity to factors that stimulate food intake.[10] Combined, these responses hinder weight loss and promote weight regain. Until recently, bariatric surgery had been the most effective treatment for maintaining a reduction in body weight[11] and, as such, is the current benchmark for anti-obesity medications.

The regulation of body weight is orchestrated primarily by the brain and adipose tissue (Figure 1), which constantly integrate information related to the body's energetic state to adjust food intake, satiety, and energy balance.[8,10] Notable hormones implicated in this gut-brain-fat communication axis include, among many others, the adipokines leptin and adiponectin, the liver-secreted hormone fibroblast growth factor 21 (FGF21), the pancreatic α cell-derived hormone glucagon, the gastrointestinal system peptides ghrelin, peptide YY (PYY), and cholecystokinin (CCK), in addition to the incretins glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP).[8]

Glucagon, traditionally known for its ability to counteract hypoglycemia, has been implicated in the pathogenesis of type 1 and T2D [12–14] (Box 1). Glucagon acts through the glucagon receptor (GCGR), and alterations in GCGR signaling can have profound effects on glucose metabolism.[12] While lack of GCGR signaling can normalize glycemia under insulin-deficient conditions,[15,16] this effect is contingent on the presence of residual insulin.[17] Interestingly, glucagon has pleiotropic biology that

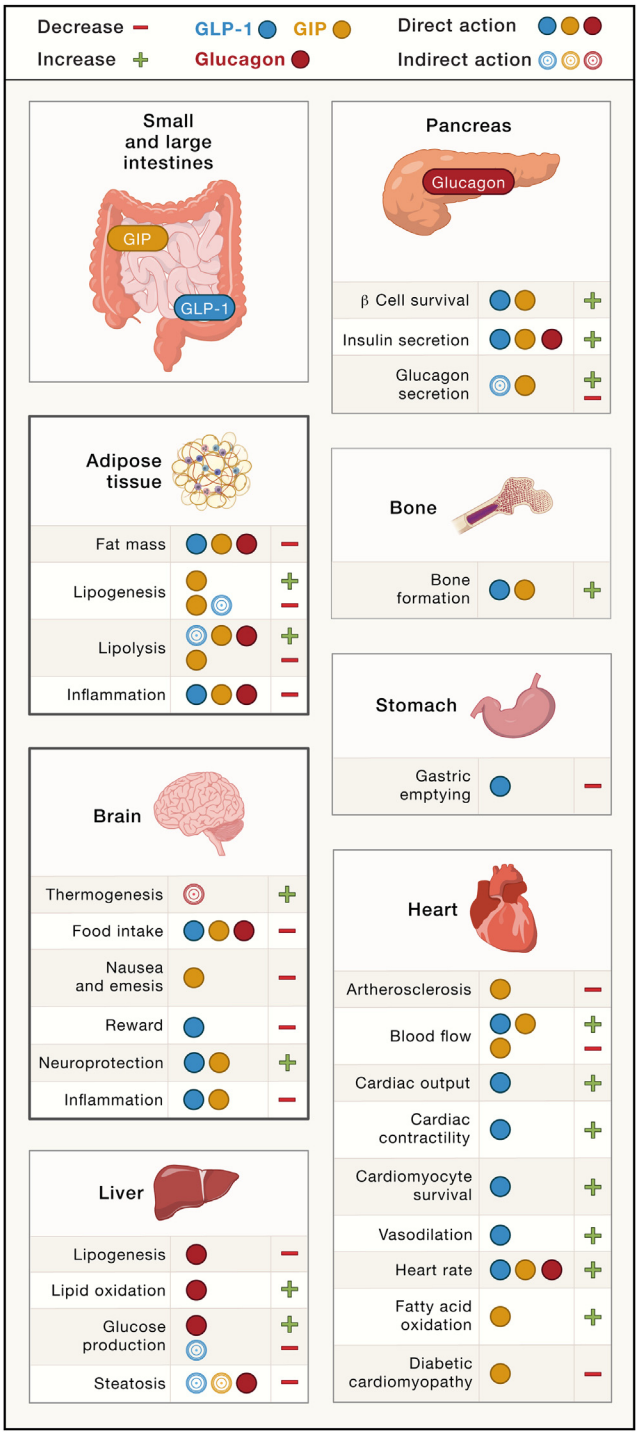

Figure 1. The signaling pathways and metabolic processes that endogenous GLP-1, GIP, and glucagon hormones act upon in target tissues

The tissue-specific metabolic effects of the endogenous gut-secreted incretin's glucagon-like peptide-1 (GLP-1) (blue circles), glucose-dependent insulinotropic peptide (GIP) (orange circles), and the pancreatic hormone glucagon (GCG) (red circles). The primary actions of GLP-1, GIP, and GCG are shown for adipose tissue, brain, liver, pancreas, bone, stomach, and heart. The bold squares highlight the brain and adipose tissue, two of the primary sites of GLP-1R agonist, GLP-1R/GIPR co-agonist, and GLP-1R/GCGR co-agonist action in the regulation of body weight. The solid circles represent the direct action of each hormone, whereas the partial circles highlight their indirect action. A red (–) sign highlights an inhibitory role for GLP-1, GIP, and GCG, whereas a green (+) sign represents a stimulatory role for each hormone. Each primary metabolic process, signaling pathway, and/or metabolic outcome that is impacted by GLP-1, GIP, or GCG, whether direct or indirect, is highlighted for each tissue.

extends beyond its role in glycemic control. In particular, preclinical studies have revealed that glucagon may be harnessed for metabolic benefits, such as body weight loss in the context of obesity.[18] Glucagon administration to rats was shown to promote a negative energy balance by increasing oxygen consumption,[18] an effect later attributed to an increase in non-shivering thermogenesis.[19] Furthermore, enhanced GCGR signaling was reported to effectively reduce body weight through inhibition of food intake[20–22] and stimulation of energy expenditure,[19,22–24] and further modulate lipid metabolism by driving lipolysis and inhibiting lipogenesis.[25–28] Glucagon also has the ability to inhibit gastric motility[29] and promote renal glomerular filtration,[30] with notable effects evident on the cardiovascular system to increase heart rate, cardiac contractility, and cardiac output[31] (Figure 1). Collectively, this supports the prospect that GCGR agonism may be employed as a viable option for the treatment of metabolic diseases associated with obesity, particularly when used in adjunct to therapeutics that are capable of restraining glucagon's acute glycemic and cardiovascular limitations.

Gut-hormone-derived GIP, secreted from enteroendocrine K cells in the upper intestine in the duodenum and jejunum mucosae,[70] was initially discovered to play an integral role in the body's response to glucose intake[37,71] (Box 1). GIP exerts a significant role in adipose tissue blood flow[72] and, under conditions of hyperinsulinemia, promotes lipid deposition in adipocytes by stimulating lipoprotein lipase.[73,74] The gut hormone also potentiates insulin-induced glucose uptake, which leads to an increase in lipid conversion from glucose.[75–77] However, *in vivo*, GIP was shown to promote lipolysis under conditions of normo- or hypoinsulinemia, and mice overexpressing GIP exhibit lower fat mass when fed a high-fat diet.[78] Preclinical studies further revealed that chemogenetic activation of GIPR neurons in either the hypothalamus or the hindbrain reduces food intake[79,80] (Figure 1) and that central GIPR agonism ameliorates the emetic effect of GLP-1 receptor (GLP-1R) agonism[81] and stimulates weight loss through inhibition of food intake.[82,83] In fact, GIP-driven weight loss is synergistically enhanced by adjunct GLP-1R agonism,[84–86] and while GIP-mediated weight loss is fully preserved in *Glp1r*-deficient mice,[82,86] GIP fails to alter food intake and body weight in mice harboring a deletion of *Gipr* in either the central nervous system (CNS)[82] or, more specifically, γ-aminobutyric acid (GABA)ergic neurons.[83]

The other incretin, GLP-1, secreted from enteroendocrine L cells in the ileum and colonic mucosae of the large intestine,[87] was identified for its role in stimulating insulin, inhibiting glucagon secretion, and gastric motility, both essential effects in the regulation of glucose homeostasis[56–58] (Box 1). In fact, studies have shown that the inhibition of glucagon secretion by GLP-1 is equally as important as enhanced insulin secretion in controlling glucose levels in T2D.[59] Beyond its role in glucose

Box 1. Discovery of the incretin hormones and the metabolic action of glucagon

The intestine has long been recognized as a key player in regulating glucose metabolism. In 1906, it was discovered that intestinal mucosal extracts decrease glucosuria in subjects with type 2 diabetes (T2D)[32] and that the excursion of blood glucose is much greater when glucose passes through the gut relative to intravenous infusion.[33] Attributed to enhanced glucose-stimulated insulin secretion,[34–36] this pointed to the intestine as the origin of insulinotropic hormones, which were subsequently identified as the glucose-dependent insulinotropic polypeptide (GIP) in 1973[37] and glucagon-like peptide-1 (GLP-1) in 1987.[38–41] GIP was initially termed gastric-inhibitory polypeptide due to its inhibition of gastric acid secretion at supraphysiological doses.[42]

In 1980, a study found that the insulinotropic effect of orally ingested glucose was diminished in subjects with Crohn's disease who underwent ileal re-section.[43] Since the impaired incretin response was not related to glucose-induced GIP secretion, it was hypothesized that the lower intestine was likely to harbor an additional GIP-like insulinotropic hormone.[43] In 1983, Creutzfeldt further reported a partially preserved incretin effect when GIP from rat intestinal extracts was neutralized,[44] while Habener discovered that the anglerfish preproglucagon cDNA encodes a novel sequence with considerable sequence homology to glucagon.[45–47] Subsequently, two glucagon-like peptides, GLP-1 and GLP-2, were identified in the hamster,[48] human,[49] and rat[50,51] proglucagon sequences. Since glucagon[52] and GIP[37] were both known to stimulate insulin secretion, it was hypothesized that the newly identified peptides may also promote insulin secretion.[45–47] Proglucagon was shown to produce several shorter forms of GLP-1, and while neither GLP-2 nor the full-length GLP-1 stimulated insulin secretion, two N-terminal truncated forms of the peptide, GLP-1 (7–37) and GLP-1 (7–36NH_2), displayed insulinotropic action in perfused pancreata from rats[39] and pigs,[38] in addition to the rat insulinoma β cell line RIN 1046.[40] The identification of different forms of the peptide,[53] as well as correlation studies on the insulinotropic activity of the different forms of GLP-1 in the isolated perfused rat pancreas, by Mojsov, contributed to establishing GLP-1 as an incretin hormone,[39] soon after being confirmed in humans.[41] GIP and GLP-1 were thus established as the predominant incretin hormones.[54,55] GLP-1 was later also discovered to affect glucose metabolism through inhibition of glucagon secretion and gastric motility.[56–58] The relevance of these non-insulinotropic GLP-1 effects was revealed in glucose clamp studies in a T2D setting, where inhibition of glucagon secretion proved equally important in glycemic control as enhanced insulin secretion.[59]

Glucagon was discovered in 1923 from pancreatic homogenates during refinement of insulin purification.[60] Five decades later, during which glucagon was chemically characterized and biologically established as an insulin counter-regulatory hormone, glucagon was still granted little to no pharmacological value beyond its ability to rescue acute hypoglycemia.[30] In fact, glucagon was purported as a caustic element in the pathogenesis of diabetes.[12] Seemingly consistent with this was the observation that glucagon receptor (*Gcgr*)-deficient mice are protected from streptozotocin-induced diabetes.[15,16] Subsequent studies, however, revealed that residual insulin is critical for normalization of glycemia in streptozotocin-treated *Gcgr*-deficient mice,[17] and further, type 1 diabetes (T1D) develops rapidly in experimental animals following surgical removal of the pancreas.[61,62] Collectively, this indicated that T1D originates from lack of insulin rather than the excess of glucagon. Nonetheless, the quest for glucagon antagonism as a potential anti-hyperglycemic therapy evolved and was supported by near-normalization of glycemia following pharmacological,[63] or genetic[64] inhibition of GCGR signaling in insulin-deficient rodents. The beneficial glycemic effects of GCGR signal inhibition are also reported from clinical studies in subjects with T2D.[65,66] Although GCGR antagonism in mice often results in pancreatic α cell hyperplasia, no such effect is observed in non-human primates.[67] On the other hand, there are persistent concerns that GCGR antagonism may increase total and low-density lipoprotein cholesterol levels.[68,69] Ironically, while GCGR antagonism has nowadays fallen from favor as obesity emerged as a prominent confounding feature of T2D, fatty liver disease, and atherosclerosis, we have witnessed a recent renaissance in utilizing GCGR agonism, predominantly in unimolecular formulations with GLP-1R agonism, for the treatment of exactly these metabolic diseases.

metabolism, GLP-1 is known to possess cardio- and neuroprotective effects, reduce cellular apoptosis and inflammation, and modulate reward behavior and palatability[8,88] (Figure 1). Furthermore, GLP-1 exerts a significant effect on body weight by inhibiting food intake through centrally mediated mechanisms.[8] The latter aspect ignited tremendous interest in GLP-1 and, along with GIP, placed them both on a trajectory as promising candidates to use for the treatment of obesity.

The physiological action of the GLP-1/GIP axis hence rendered these gut hormones as attractive medicinal targets to treat T2D and, subsequently, obesity. In particular, GLP-1R agonism not only emerged as a powerful tool in the treatment of T2D and excess adiposity[89] but also displayed favorable effects on the cardiovascular system[90] and neurodegenerative diseases.[88] This highlights that GLP-1R agonists have an appreciable action profile outside their original targets in the pancreas.[88] The brain does not rely on a single factor to regulate energy metabolism; rather, it integrates a variety of independent signals to adjust energy intake and energy expenditure.[10] Similarly, the adipocyte is at the frontline of caloric reserves, caloric influx, and energy expenditure and, as such, is well positioned to orchestrate systemic responses through multiple signals.[91,92]

Consequentially, a pharmacotherapy that engages multiple key metabolic signals would be expected to achieve greater weight loss relative to a drug that targets only one metabolically relevant signaling pathway. Consistent with this model is a battery of preclinical and clinical studies demonstrating that weight loss induced by GLP-1R agonism is enhanced when adjunctively administered with glucagon, GIP, amylin, CCK, or FGF21, which can be achieved as co-therapy, or in a unimolecular polyagonist formulation.[8] Such poly-pharmacotherapies are designed to allow for individual sub-maximal dosing at each target receptor and may therefore not only optimize weight loss through complementary pharmacology at several independent receptors but also improve tolerability while dampening the likelihood of tachyphylaxis. The recent emergence of unimolecular polyagonists constitutes a forefront in next-generation drugs for the treatment of metabolic diseases, such as obesity.[24,93] Drugs possessing GLP-1R agonism with complementary pharmacology through the GIPR have yielded astonishing weight loss efficacy, concomitant with an excellent safety profile, and superior metabolic outcomes relative to the best-in-class GLP-1R agonists. Supplemental pharmacology through the glucagon receptor, either in unimolecular combination with GLP-1R or added to GLP-1R/GIPR co-agonism, is

currently setting a new benchmark. These latest-generation agents not only accelerate further weight loss but also address obesity-associated co-morbidities, such as fatty liver disease, cardiovascular disease, and dyslipidemia. Here, we discuss the turbulent path and controversies that span decades leading to the integration of GLP-1, GIP, and glucagon as synergistic partners in cutting-edge incretin-based unimolecular therapeutics, highlighting the instrumental clinical achievements to date.

THE DEVELOPMENT OF GLP-1R AGONISTS IN THE TREATMENT OF T2D AND OBESITY

The identification of GLP-1 as an insulinotropic hormone, along with the demonstration that the incretin has beneficial effects well beyond its action on the pancreas (Figure 1), spurred great interest to explore its pharmacological potential for the treatment of T2D and obesity. However, the pharmacological use of native bioactive GLP-1 is limited by an exceedingly short half-life (~2–3 min), resulting from rapid renal elimination and proteolytic degradation by dipeptidyl peptidase-4 (DPP-4)[94] and neutral endopeptidase 24.11.[95] It is estimated that as little as 10% of active GLP-1 reaches the general circulation, and only a mere fraction of this reaches the brain.[96] Nonetheless, continuous infusion of GLP-1,[97] or repeated administration of DPP-4 inhibitors,[98] improves glucose metabolism in subjects with T2D.

The pharmacokinetic limitations of native GLP-1 have been improved by a variety of chemical modifications, which serve to enhance the pharmacology of the hormone through increased molecular stability, enhanced plasma concentration, and delayed renal clearance. Several selective GLP-1 analogs have received regulatory approval in the last decade and include formulations suitable for twice daily (exenatide), daily (liraglutide, lixisenatide), and weekly (exenatide extended-release, albiglutide, dulaglutide, and semaglutide) subcutaneous injections. More recently, the development of a daily orally administered form of semaglutide has been introduced as a promising alternative to weekly injections.[99] While these peptides are highly efficacious for the treatment of T2D, as a drug class, they all demonstrate transient dose-dependent gastrointestinal adverse effects, such as nausea and vomiting, which require a carefully orchestrated dose escalation to reach maximal effects.

Liraglutide (3 mg) was the first GLP-1R agonist registered for the treatment of obesity. This GLP-1R agonist was initially approved for the treatment of obesity in adults, then later for obesity in children and adolescents. In subjects with obesity without T2D, following 1 year of treatment with liraglutide, ~5.2% placebo-corrected weight loss was achieved, with approximately a third of subjects reaching weight loss of >10%.[100] In this study, the body weight reduction induced by liraglutide was associated with improved glucose control, a decrease in systolic and diastolic blood pressure (−2.8 and −0.9 mmHg over placebo controls), along with an improvement in lipid and cholesterol profiles, albeit with a slight increase in heart rate of 2.4 beats/min in comparison with placebo-treated controls.[100] In 2021, the United States Food and Drug Administration (FDA) approved once weekly (OW) semaglutide (2.4 mg) for the treatment of obesity. Following 68 weeks of treatment, semaglutide impressively lowered body weight in non-diabetic, obese individuals by 14.9%, relative to 2.4% in placebo-treated controls.[101] By contrast, semaglutide was less efficacious in subjects with obesity and T2D, with placebo-corrected weight loss of only 6.2% reported with 68 weeks of treatment.[102]

Nevertheless, these results highlight the precedent-setting ability of GLP-1R agonists, when properly dose titrated, to meaningfully reduce body weight. They further firmly set the foundation to achieve greater weight loss that most obese individuals require with pharmacological means and, as such, raise the critical question as to whether additional weight loss can be reached with complementary pharmacology.

GUT-HORMONE MULTI-RECEPTOR AGONISTS FOR THE TREATMENT OF T2D AND OBESITY

Unimolecular multi-receptor agonists that employ several independent signaling pathways are emerging as the best-in-class drugs for glycemic control and weight loss. The pleiotropic nature of glucagon's biology,[30] together with a rekindled interest in the pharmacology of GIP, has ignited much interest in exploring their therapeutic use in unimolecular formulations with GLP-1R agonism to treat obesity and diabetes. The objective has been to increase the magnitude of weight loss possible in a broad community of individuals with obesity, without imposing safety limitations that naturally reside in GLP-1R agonism. Particularly in subjects with obesity and persistent T2D, GLP-1R-driven weight loss still plateaus in the single-digit range,[102] and as such, enhanced efficacy from supplemental pharmacology to further accelerate weight loss, while ideally simultaneously addressing obesity-linked co-morbidities, remains the primary goal. Multiple gut-hormone combinations have been explored preclinically, with an appreciable number having advanced to clinical studies, with unimolecular peptides possessing varying degrees of GLP-1R, GIPR, and GCGR activity constituting the clinically most matured set of drug candidates. Here, we will discuss the preclinical and clinical studies in receptor co-agonism of GLP-1 with GIP or glucagon, as well as the fully integrated triagonists.

GLP-1R/GCGR co-agonists

In recent years, there has emerged a deeper appreciation for the non-pancreatic biology of glucagon, specifically anchored on its role as a weight-regulatory hormone in energy balance and satiety.[30,103,104] In diet-induced obese rodents, GCGR agonist administration drives weight loss through a reduction in food intake, an induction of lipid utilization via brown fat thermogenesis, along with an increase in whole-body energy expenditure; the latter effect in part attributed to the stimulation of liver-secreted FGF21 and transcriptional upregulation of the hepatic bile acid-activated nuclear receptor, farnesoid X receptor.[19,30,105,106] This partial regulation in energy expenditure suggests that there could be room for additional factors and mechanisms by which enhanced GCGR signaling mediates energy balance. Interestingly, treatment of diet-induced obese mice with a GCGR agonist revealed that glucagon-stimulated energy expenditure and weight loss can also be driven by hepatic amino acid catabolism and thus a systemic response to hypoaminoacidemia.[107] In addition to the liver, preclinical studies further

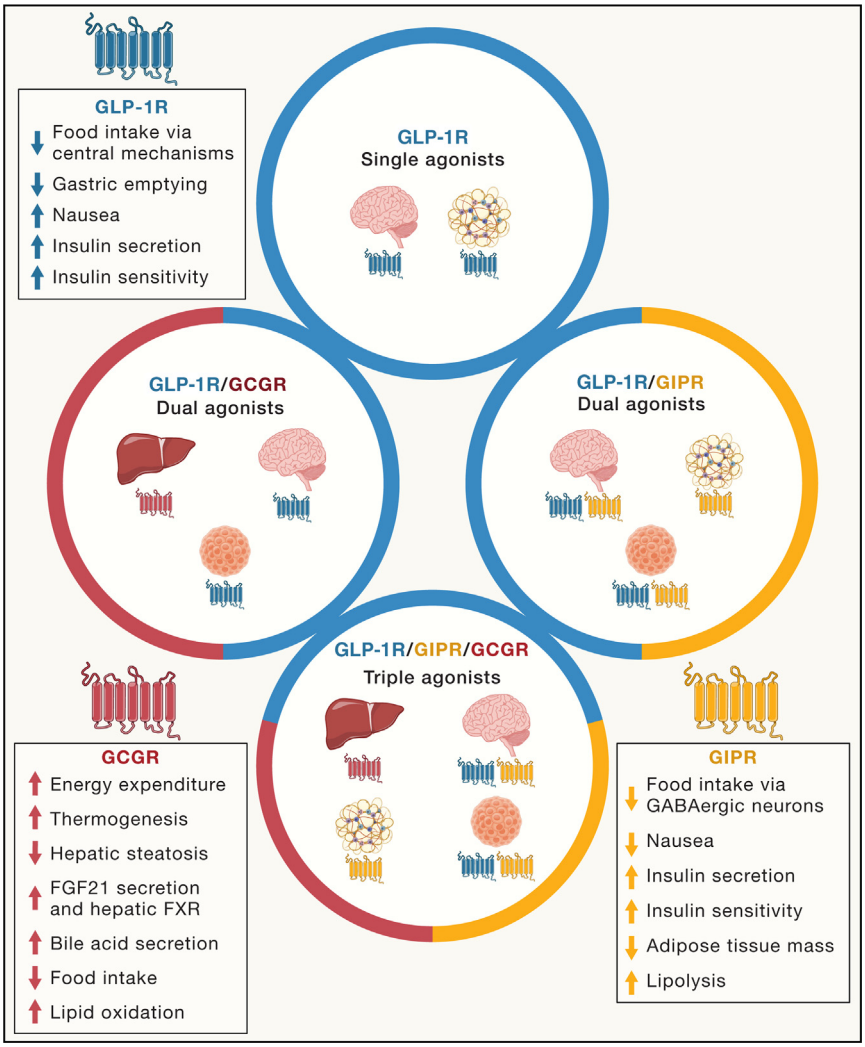

Figure 2. The main sites and tissue-specific action of GLP-1R mono-agonists, GLP-1R/GCGR co-agonists, GLP-1R/GIPR co-agonists, and GLP-1R/GIPR/GCGR triagonists determined in preclinical studies

The primary sites of receptor expression and action and the pharmacological effects mediating body weight loss and improvements in glycemic control of GLP-1R agonists (blue text and circle), GLP-1R/GCGR co-agonists (blue/red text and circle), GLP-1R/GIPR co-agonists (blue/yellow text and circle), and GLP-1R/GIPR/GCGR triagonists (blue/yellow/red text and circle). Preclinical studies have reported the metabolic effects, signaling pathways, and potential key regulators that each mono- or multi-receptor agonists target in the brain, pancreatic islets, adipose tissue, the liver, and the kidney. Graphics were created with BioRender.com.

suggest a role for GCGR agonism in the kidney.[108] The GCGR is potently downregulated during chronic kidney disease, and the lack of glucagon signaling in the kidney renders the tissue susceptible to fibrosis, inflammation, oxidative stress, and lipid accumulation.[108] This indicates that some of the cardiorenal benefits observed for co-agonists may exert their beneficial effects directly through a kidney-GCGR signaling axis.

Together with an appreciable (∽50%) sequence homology with the incretin hormones,[109] the non-glycemic effects of glucagon thus render the peptide an attractive candidate for a unimolecular liaison with GLP-1 and GIP. Importantly, the benefits of such polypharmacotherapy are tethered on the assumption not only that these agents would bolster weight loss efficacy through complementary pharmacology at each target receptor but also that the positive glycemic and cardiovascular effects of the incretins would restrain any potentially detrimental effects that may, or may not, reside in GCGR agonism. Unimolecular peptides of GLP-1R and the GCGR were the first purposeful co-agonists to emerge, seeking to amalgamate the glucagon-mediated increase in energy expenditure with the anorectic action of GLP-1R to promote weight loss, while employing the anti-diabetic action of GLP-1 to minimize the diabetogenic risk of unopposed glucagon receptor agonism.[110]

Once proven that full potency at each of the two receptors could be chemically assembled into a single chimeric peptide of comparable size to each native hormone, studies in animal models of obesity, utilizing first-generation GLP-1R/GCGR co-agonists, resulted in superior weight loss, enhanced glucose-lowering efficacy, and a reduction in food intake when compared with selective GLP-1R agonists[111–114] (Figure 2). These initial preclinical reports thus promoted an avalanche of pharmaceutical interest that validated these initial observations to a point where numerous GLP-1R/GCGR peptides have now progressed from bench-to-clinical development, with the most characterized described below.

The first GLP-1R/GCGR co-agonist to emerge

The first preclinically evaluated GLP-1R/GCGR co-agonist was based on the glucagon sequence, in which amino acid residues from GLP-1 and GIP were stepwise introduced to achieve balanced activity at both target receptors.[112] The DPP-4-protected peptide carried a 40 kDa polyethylene glycol to delay renal clearance, and after weekly dosing in diet-induced obese mice, the peptide lowered body weight and reduced fat mass by ∼25.8%, achieving these effects through the synergistic anorectic action at both target receptors, with complementary thermogenic and lipolytic effects attained through GCGR agonism.[112] The more recently developed SAR425899 employs elements of glucagon in the sequence of exendin-4 to provide GCGR agonism while maintaining GLP-1R activation.[115] SAR425899 was shown to effectively reduce body weight and fat mass in obese mice, stimulate robust glycemic effects in leptin receptor-deficient diabetic *db/db* mice and, remarkably, reduce total caloric intake and increase energy expenditure in

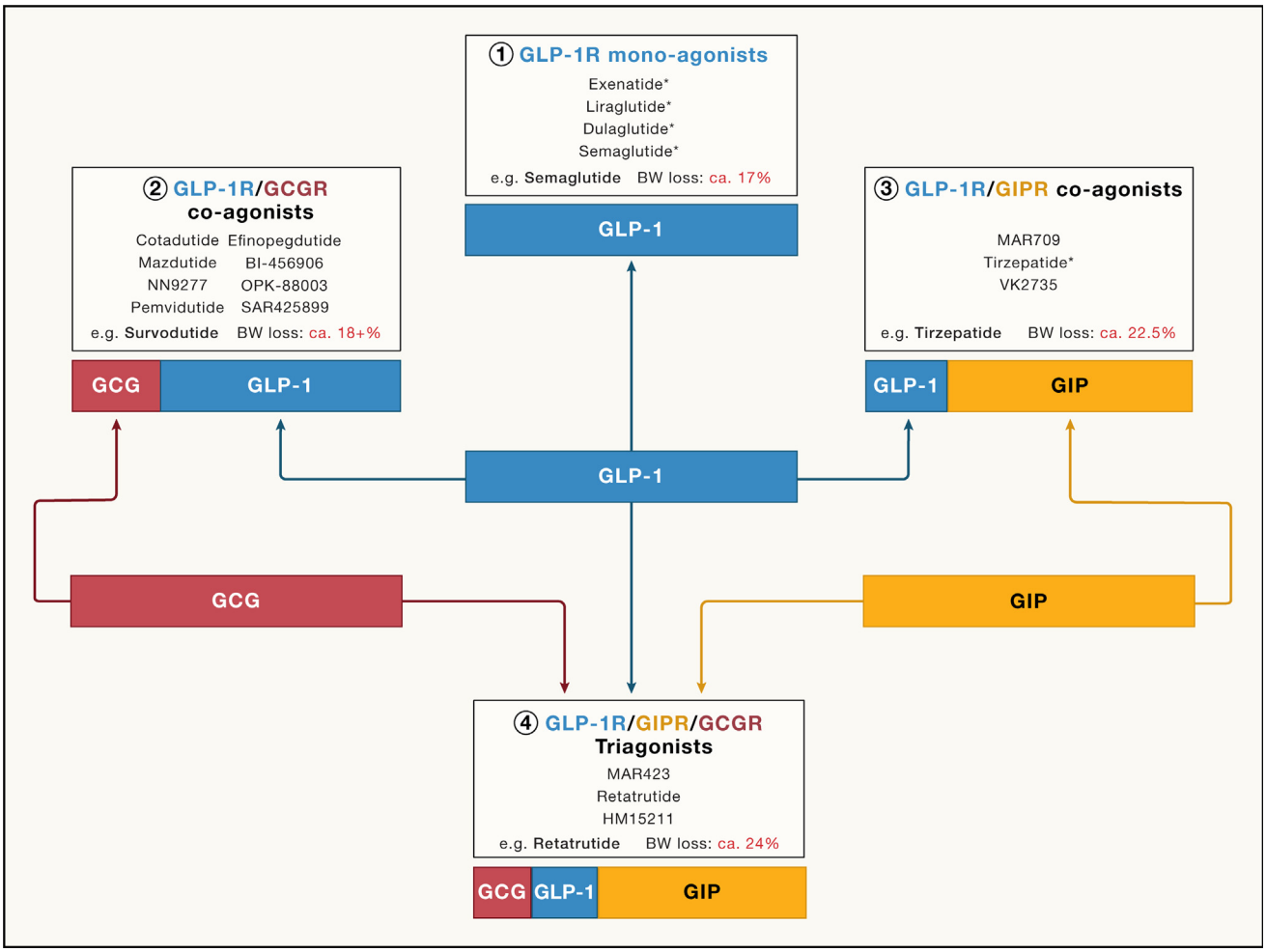

Figure 3. The mono- and unimolecular multi-receptor agonists that have completed or are currently in ongoing clinical trials

Peptide-based therapeutics based on (1) GLP-1R single agonists compared with unimolecular (2) GLP-1R/GCGR co-agonists, (3) GLP-1R/GIPR co-agonists, and (4) GLP-1R/GIPR/GCGR triagonists that have completed clinical studies or are currently being examined in clinical trials. The blue boxes and arrows highlight GLP-1, the orange boxes and arrows represent GIP, and the red boxes and arrows show glucagon (GCG), and the corresponding ratio of each hormone receptor in each multi-receptor agonist peptide. All co-agonists and triagonists have displayed beneficial metabolic effects in lowering HbA_{1c} levels and reducing body weight (BW) for the treatment of T2D and obesity. Examples of each category and their relative contributions of the respective ligands are displayed, adjacent to the percent BW loss (red text) reported in their corresponding clinical trials. *FDA-approved peptides. Graphics were created with BioRender.com.

obese diabetic non-human primates.[113,116] In a phase 2 study composed of obese subjects with T2D, SAR425899 outperformed liraglutide after 26 weeks of treatment to yield greater improvements in postprandial glycemic control, along with superior pancreatic β cell responsiveness and enhanced insulin sensitivity.[117] In a more recent phase 1b study in overweight and obese subjects, treatment with SAR425899 for 19 days reduced body weight and increased lipid oxidation relative to subjects receiving a calorie-restricted diet, with such effects being notably consistent with the thermogenic capacity of glucagon to enhance energy expenditure.[118] This was the first human trial to demonstrate that GLP-1R/GCGR co-agonism could be a suitable option for weight loss maintenance and added clinical validation that glucagon agonism could significantly contribute to the regulation of energy balance in an obese setting (Figure 3). Nonetheless, the clinical development of SAR425899 was eventually discontinued due to adverse effects, primarily nausea and vomiting.

Mazdutide

Mazdutide (also termed IBI362, oxyntomodulin 3 [OXM-3], or LY3305677) is a single-chain synthetic GLP-1R/GCGR co-agonist analogous to mammalian OXM and modified with a fatty acyl side chain to extend its circulating half-life.[119] In diet-induced obese mice, mazdutide was reported to lower body weight, improve glycemic control, and increase energy expenditure, with partially preserved action evident in mice harboring a deletion of either *Glp1r* or the *Gcgr*.[120] The peptide further reduced food intake and improved glucose tolerance in both diet-induced obese mice and streptozotocin-induced diabetic mice.[120] Additional metabolic benefits of mazdutide treatment include, but are not limited to, increasing systemic levels of FGF21 and lowering plasma triglyceride levels.[120] These

properties render mazdutide a highly effective co-agonist to treat the metabolic dysfunction associated with obesity, particularly through the indirect stimulation of FGF21 action, one of the key endocrine FGFs noted for its ability to effectively lower systemic triglyceride levels in humans.[121] A recent phase 2 study, composed of 248 overweight and obese Chinese subjects, documented a body weight reduction of ~11.3% from baseline following 24 weeks of mazdutide treatment at the highest dose.[122] The peptide was further shown to lower blood pressure; reduce lipid, blood uric acid, and transaminase levels; and alleviate hepatic lipid accumulation.[122] Mazdutide is currently being investigated in the phase 3 DREAM and GLORY trials for the treatment of obesity (NCT05607680) and T2D (NCT05606913).

As a general note, it is essential that clinical trial cohorts are recruited from diverse ethnic backgrounds. Well-established differences in diabetes prevalence, the incidence of metabolic complications, and death rates occur among different ethnic groups. Black and Hispanic individuals exhibit a higher burden of T2D[123] and higher rates of diabetic complications, such as cardiovascular disease. In fact, cardiovascular disease mortality rates are among the highest in Black T2D individuals. It is therefore essential to better understand what the differential disease susceptibilities translate to, particularly with respect to varying metabolic responses to the co-agonist peptides in populations of different ethnic backgrounds.

Cotadutide

Cotadutide (also termed MEDI0382) is a synthetic peptide based on human OXM, which employs a palmitic acid side chain[124] that enables albumin binding and thus extension of its duration of action. The peptide is notably more potent at GLP-1R, relative to GCGR, with a ratio of ~5:1.[125] Similar to other GLP-1R/GCGR co-agonists, cotadutide displays robust metabolic efficacy in rodents and healthy cynomolgus non-human primates, with superiority in weight loss and glucose control, when compared with the selective GLP-1R agonist liraglutide.[125] This enhanced body weight-lowering efficacy is again attributed to a GCGR agonist-driven increase in energy expenditure, associated with a GLP-1R agonist-mediated induction in satiety.[125] More recently, cotadutide was shown to alleviate hepatic steatosis, dampen liver fibrosis, and improve mitochondrial function in murine models of metabolic dysfunction-associated steatotic liver disease (MASLD), far more effectively than liraglutide.[126] Of note, to raise disease awareness and prevent stigma, the terminology and diagnostic criteria of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) were recently replaced with the nomenclature MASLD and metabolic dysfunction-associated steatohepatitis (MASH), respectively.[127] Taken together, cotadutide and similar co-agonists are firmly positioned as viable prospects for the treatment of MASLD and MASH. Moreover, their metabolic benefits signal that enhanced glucagon agonism can promote hepatic de-lipidation, which should have vast medicinal advantages for the treatment of obesity, independent of the magnitude of weight loss. In clinical studies, cotadutide was indeed well tolerated,[128,129] and in phase 2 clinical trials, the peptide displayed impressive weight loss efficacy, superior hepatic lipid-lowering capabilities, and appetite suppression, along with a reduction in blood pressure and HbA_{1c} levels in individuals with obesity and T2D.[124,128,130] Following 32 days of daily treatment, cotadutide further improved postprandial glucose control and potentiated weight loss in subjects with T2D and chronic kidney disease, which was paralleled with a notable 51% reduction in the urinary albumin-to-creatinine ratio.[131] Cotadutide is currently being assessed in phase 2b clinical trials in subjects with MASLD (PROXYMO-ADV, NCT05364931), with several additional phase 2b studies completed in subjects with either (1) chronic kidney disease with T2D (NCT04515849), (2) obesity and MASLD/MASH (NCT04019561), or (3) obesity with T2D (NCT03555994), with results awaiting publication (Figure 3).

Other GLP-1R/GCGR co-agonists

NN9277/NN6177 (also termed NN-117/NNC9204-1177) was developed by Novo Nordisk for the treatment of obesity and T2D.[132] NN9277 completed three phase 1 clinical trials in healthy subjects, in addition to overweight and obese subjects, utilizing multiple doses ranging from 1–6 mg (NCT04059367, NCT03308721, and NCT02941042). In obese subjects, NN9277 displayed impressive weight loss efficacy, with a placebo-adjusted reduction in body weight of ~12.6% following 12 weeks of treatment.[132] However, its clinical development was eventually discontinued due to adverse effects, most notably increased heart rate and impaired glucose tolerance.[132]

ALT-801, previously known as SP-1373, is a potent, OW-balanced co-agonist for GLP-1R and GCGR. The peptide was chemically derivatized with a glycolipid through the use of surfactant-peptide conjugation technology to delay absorption and further extend its half-life.[133] This co-agonist was initially designed for the treatment of MASLD and obesity, which is supported by the observation that after 12 weeks of peptide administration in a mouse model of obesity and MASLD, a reduction in body weight by ~25% was apparent, concomitant with an amelioration of hepatic steatosis, inflammation, and fibrosis.[133] Much like cotadutide and the broader family of glucagon-based multi-receptor agonists, these results encourage a promising avenue for ALT-801 in the treatment of obesity-associated MASLD. In fact, ALT-801, now termed pemvidutide, is being examined in a clinical setting,[133] as five trials either are ongoing or have been completed, all in the context of obesity, T2D, or MASLD. In a phase 1 study composed of 100 overweight and obese subjects, 12 weeks of treatment with pemvidutide (1.8 mg) resulted in placebo-corrected weight loss of ~10.3% (NCT04561245). Pemvidutide is currently also being assessed for the treatment of obesity in a 48-week phase 2 multiple-ascending-dose study, composed of 320 obese subjects (MOMENTUM obesity trial) (NCT05295875).

Another GLP-1R/GCGR co-agonist, JNJ-64565111 (also termed HM-12525A, efinopegdutide, or MK-6024), was assessed in several phase 2 clinical studies for the treatment of obesity[134,135] and T2D[135] (NCT03586830). In subjects with obesity without T2D, 26 weeks of treatment with JNJ-64565111, at the highest tested dose of 10 mg, resulted in a placebo-corrected weight loss of ~10%, relative to −5.8% in liraglutide-treated controls.[134] In subjects with obesity and T2D, 12 weeks of JNJ-64565111 treatment reduced placebo-corrected body weight by ~7.2%, however without notable effects on HbA_{1c} levels or fasting plasma glucose levels, albeit with an appreciable reduction in fasting insulin levels.[135] In both studies, treatment

with JNJ-64565111 resulted in an increased appearance of adverse effects, most notably nausea and vomiting.[134,135] The frequency of these side effects occurred in 84% and 67% of subjects receiving JNJ-64565111 and in 71% and 48% of subjects administered liraglutide.[134] JNJ-64565111 is currently being evaluated in comparison with semaglutide in a phase 2 study in subjects with MASLD (NCT04944992). Two additional GLP-1R/GCGR co-agonists are currently being examined in clinical trials; however, their metabolic outcomes await publication. In particular, BI-456906 (also termed survodutide) is a fatty-acylated peptide that is being evaluated in phase 2 trials for the treatment of obesity (NCT04667377) and T2D (NCT04153929). Additionally, the co-agonist peptide OPK-88003 is being explored in a phase 2 study in subjects with T2D (NCT03406377).

In summary, an abundance of preclinical and clinical (Figures 2 and 3) studies actively focus on GLP-1R/GCGR co-agonists, with the majority of peptides displaying an impressive impact on reducing body weight, improving glycemic control, and maintaining or restoring metabolic homeostasis. It will be intriguing to follow how these particular GLP-1R/GCGR co-agonists will compete in the arena with other incretin-based co-agonist combinations, in addition to the superiority of the rapidly imminent triagonist peptides in the near future. Additional questions center on whether the combination of three target receptor agonists will prove advantageous with respect to metabolic improvements in cardiorenal or hepatic function. Moreover, the question is what will prove the most suitable ratio of relative receptor affinities for either co-agonist or triagonist peptides, which will translate to minimally associated adverse effects?

GLP-1R/GIPR co-agonists

The rationale for combining GLP-1R and GIPR agonism

Anchored on the lingering observation that germline *Gipr*-deficient mice are protected from diet-induced obesity,[136] combined with the findings that show the insulinotropic action of GIP is largely dampened in subjects with T2D,[137] there is persistent debate as to whether the receptor should be activated or inhibited to reach optimal metabolic merit.[138–140] For instance, pharmacological or genetic inhibition of the GIPR has been shown to alleviate intramuscular lipid accumulation in aged mice.[141] Furthermore, while some *GIPR* mutations are associated with a lower body mass index in humans,[142–144] certain GIPR antagonists reduce body weight and caloric intake in diet-induced obese mice and non-human primates, particularly when given in adjunct to GLP-1R agonism.[145,146] However, while protection from obesity is also observed in *Glp1r*-deficient mice,[147,148] near-normalization of hyperglycemia restores the insulinotropic effect of GIP in subjects with T2D.[149] By contrast, GIPR agonist treatment is equally effective in promoting weight loss in obese mice, and further, GIP-driven weight loss is also synergistically enhanced by adjunct GLP-1R agonism.[84–86,150] Importantly, in mice with loss of the *Gipr* in the CNS at large, the GIP-driven inhibition in food intake is completely diminished, while the GIP-induced weight loss is partially restored.[82] This indicates that GIP drives weight loss via central inhibition of food intake and through non-CNS peripheral mechanisms unrelated to food intake. Additional evidence for the peripheral benefits of GIPR activation stem from recent observations showing that the anti-inflammatory effects of GLP-1R activation are mediated exclusively through the central actions of GLP-1R.[151] However, treatment of brain-specific *Glp1r*-deficient mice with a GLP-1R/GIPR co-agonist retains anti-inflammatory action, suggesting that peripheral GIPR activity in adipose tissue and immune cells, i.e., macrophages, can elicit positive systemic effects.[151] In light of this, we would postulate that peripheral activation of the GIPR in white adipocytes has the capacity to modulate energy balance and weight loss. To substantiate these claims, further work with a focus on adipose tissue is warranted in the future to better define the contributions that peripheral GIPR activation, specifically in the white adipocyte, elicits toward the overall metabolic benefits of these agonists.

The rationale for engaging GIPR agonism in unimolecular liaison with GLP-1 was thus 2-fold. First, combining GLP-1R and GIPR agonism could further improve glucose metabolism through additive insulinotropic and glucagonostatic action on the pancreas and potentially even restore the impaired GIPR sensitivity characteristic of T2D.[152] Second, the physical combination of GLP-1 and GIP could synergize to outperform GLP-1-based mono-agonism, thus yielding greater weight loss with a further reduction in food intake.[85] While the question of the therapeutic value of GIP has been hampered by the ongoing debate as to whether GIPR should be activated or inhibited,[138–140,153] no GIPR antagonist has yet received regulatory approval, and the success of GLP-1R/GIPR co-agonism, as discussed below, has rehabilitated the opinion that GIPR agonism is a successful constituent of incretin-based therapies.

There are two notable unimolecular GLP-1R/GIPR co-agonist peptides that have been preclinically and clinically most characterized to display enhanced glucose-lowering potential, superior weight loss, and appetite suppression when evaluated with comparable GLP-1R agonists. They are NN0090-2746 (also known as NN9709, MAR709, RG7697, or RO6811135),[82,85] and tirzepatide (initially referred to as LY3298176)[84,154–156] (Table 1). Preclinical studies focused on whether the addition of GIPR activation enhances or provides unique metabolic benefits through mechanisms distinct from GLP-1R mono-agonism.

Discovery of the first GLP-1R/GIPR co-agonist

Spanning a decade, there have been milestone achievements in the discovery of GLP-1R/GIPR co-agonists, which were spring boarded from the foundation of GLP-1R agonists. In 2013, ahead of the curve of multi-receptor drug discovery for the treatment of obesity, the first GLP-1R/GIPR co-agonist was generated, as a single peptide with potent, balanced co-agonism at GLP-1R and GIPR.[85] This peptide was built on a glucagon sequence that was chemically modified to a peptide with comparable agonism at the GLP-1R and GIPR but devoid of GCGR activity. The first generation of GLP-1R/GIPR co-agonist peptides was pegylated to support OW dosing. This co-agonist displayed superior efficacy with anti-hyperglycemic and insulinotropic effects in diabetic *db*/*db* mice, Zucker diabetic fatty rats, and cynomolgus non-human primates.[85] In diet-induced obese mice, the co-agonist impressively reduced body weight by ~26.9%, compared with 15.6% following liraglutide treatment. The peptide further reduced food intake, decreased fat mass, and ameliorated hepatic steatosis relative to equimolar dosing with either incretin alone (Figure 2). This highlighted for the first time that

Table 1. Completed and ongoing clinical trials of the GLP-1R/GIPR co-agonists

Molecule and company	Development phase and status	Indication and duration	Primary outcomes	References and/or Clinical Trial.gov ID
MAR709 (also termed NN0090-2746 or RG7697); Novo Nordisk	phase 1	(1) 6 healthy subjects; (2) 53 T2D subjects (6 weeks)	(1) proof-of-concept study: healthy subjects (ascending doses of 4–30 mg) exhibited lowered blood glucose levels, enhanced glucose-induced insulin secretion; (2) T2D subjects: decreased HbA_{1c} by −1.11% at 30 mg dose	Finan et al.[85]
	phase 1	51 healthy subjects (single dose)	first-in-human study: ascending doses of 0.03–5 mg; dose-dependent reduction in glucose levels in response to a meal tolerance test; small increase in heart rate	Portron et al.,[157] NCT01676584
	phase 1	56 T2D subjects (2 weeks)	dose-escalation study (0.25–2.5 mg): reduced glucose levels, reduction in HbA_{1c} by −0.67% with 2.5 mg dose vs. placebo; reduced glucagon and cholesterol; small increase in heart rate	Schmitt et al.,[158] NCT01789788
	phase 2	37 T2D subjects on metformin (12 weeks)	once daily (1.8 mg) treatment of co-agonist or placebo: reduced HbA_{1c} by −0.96% and improved glycemic control and lipid parameters, relative to placebo; adverse effects observed	Frias et al.,[159] NCT02205528
Tirzepatide (LY3298176 [LY]); Eli Lilly	phase 1	total of 147 healthy or T2D subjects (26 weeks)	proof-of-concept study: first study to show the pharmacology of LY translated to clinic; in T2D subjects, LY (10 and 15 mg) reduced glucose levels, HbA_{1c}, and body weight, relative to placebo	Coskun et al.,[84] NCT02759107
	phase 2	316 T2D subjects (26 weeks)	ascending LY treatment (1–15 mg) showed superior glucose control and weight loss, dose-dependent reduction in HbA_{1c} from baseline by −1.94%, relative to the GLP-1R agonist dulaglutide	Pirro et al.,[160] NCT03131687
	SURPASS-1 (phase 3)	478 T2D subjects with obesity (40 weeks)	verified safety and efficacy: at 4 weeks of tirzepatide (5, 10, or 15 mg), HbA_{1c} robustly decreased by −1.91% (5 mg), −1.93% (10 mg), and −2.11% (15 mg) vs. placebo, with profound weight loss	Rosenstock et al.,[161] NCT03954834
	SURPASS-2 (phase 3)	2,526 T2D subjects, add-on treatment to metformin (40 weeks)	tirzepatide was superior to semaglutide, as 5, 10, and 15 mg lowered HbA_{1c} by −2.01, −2.24, and −2.30 vs. −1.86 with semaglutide (1 mg); average weight loss was doubled with tirzepatide −7.6, −9.3, and −11.2 vs. −5.7 kg with semaglutide	Frías et al.,[162] NCT03987919
	SURPASS-3 (phase 3)	502 T2D insulin-dependent subjects on metformin ± SGLT2 inhibitors (52 weeks)	tirzepatide was superior to insulin, degludec; tirzepatide (5–15 mg) lowered HbA_{1c} by −2.37 at 15 mg vs. −1.34 with insulin; reduced liver enzymes and weight loss of −15.2 kg achieved	Ludvik et al.,[163] NCT03882970
	SURPASS-3 MRI (phase 3)	296 T2D insulin-dependent subjects on metformin ± SGLT2 inhibitors (52 weeks)	a sub-population study from the SURPASS-3 trial: utilized MRI to show that tirzepatide reduced liver fat content, abdominal adipose tissue, and body weight, relative to insulin degludec	Gastaldelli et al.,[164] NCT03882970L
	SURPASS-4 (phase 3)	2,002 T2D subjects on metformin with high cardiovascular disease (CVD) risk (52 weeks)	tirzepatide outperformed insulin, glargine; tirzepatide (5–15 mg) achieved optimal glycemic control (HbA_{1c} by −2.4 at 15 mg), relative in insulin glargine (−1.4 at 100 U/mL)	Del Prato et al.,[165] NCT03730662
	SURPASS-5 (phase 3)	475 T2D subjects on insulin glargine ± metformin (40 weeks)	tirzepatide had potent anti-hyperglycemic action; tirzepatide robustly lowered fasting glucose levels (−61 to 68 mg/dL) vs. placebo (−39 mg/dL), with a −2.59% reduction in HbA_{1c}	Dahl et al.,[166] NCT04039503
	SURPASS-6 (phase 3)	1,428 T2D subjects on insulin glargine ± metformin (68 weeks)	tirzepatide compared with insulin, lispro, with a reduced in HbA_{1c} as the primary endpoint; awaiting results	Rosenstock et al.,[167] NCT04537923

(*Continued on next page*)

Table 1. *Continued*

Molecule and company	Development phase and status	Indication and duration	Primary outcomes	References and/or Clinical Trial.gov ID
	SURPASS-CVOT (phase 3)	13,299 T2D subjects with confirmed CVD (54 weeks)	assessing CVD safety and efficacy of tirzepatide vs. dulaglutide (1.5 mg); the latter having confirmed cardioprotective effects; primary readouts: changes in HbA_{1c}, myocardial infarction, stroke, and cardiovascular death	study ongoing, NCT04255433
	SURPASS-PEDS (phase 3)	90 T2D children ± metformin ± insulin (60 weeks)	pediatric population of children/teenagers (aged 10–18 years) with T2D taking metformin/insulin, treated with tirzepatide vs. placebo; primary outcomes: safety, efficacy, and HbA_{1c}	study ongoing, NCT05260021
	SURPASS-J-mono (phase 3)	636 Japanese T2D subjects (52 weeks)	tirzepatide (5, 10, and 15 mg) was superior to dulaglutide (0.75 mg); decreased HbA_{1c} by −2.4 (5 mg), −2.6 (10 mg), and −2.8 (15 mg) tirzepatide, compared with −1.3 for dulaglutide; this was greater than SURPASS 1–5 trials	Inagaki et al.,[168] NCT03861052
	SURPASS-J-combo (phase 3)	443 Japanese T2D subjects on anti-diabetic drugs (52 weeks)	tirzepatide (5, 10, and 15 mg) as an add-on to oral anti-hyperglycemic medications; body weight decreases of −5.1% (5 mg), −10.1% (10 mg), and −13.2% (15 mg); marked reductions in HbA_{1c} also observed with tirzepatide	Kadowaki et al.,[169] NCT03861039
	SURPASS-AP-combo (phase 3)	917 T2D subjects on metformin ± sulfonylurea (40 weeks)	tirzepatide was superior to insulin glargine in T2D subjects from Australia, China, India, and South Korea; weight loss achieved: −6.5% (5 mg), −9.3% (10 mg), and −9.4% (15 mg); HbA_{1c} reductions of −2.24%, −2.44%, and −2.49%, respectively	Gao et al.,[170] NCT04093752
	SURMOUNT-1 (phase 3)	2,539 overweight or obese subjects (72 weeks)	tirzepatide (5, 10, and 15 mg) caused weight loss of 15.0%, 19.5%, and 20.9%, compared with 3.1% with placebo; total body-fat mass was reduced by 33.9%, compared with 8.2% with placebo	Jastreboff et al.,[171] NCT04184622
	SURMOUNT-2 (phase 3)	938 T2D subjects overweight/obese (72 weeks)	at 72 weeks, tirzepatide (10 or 15 mg) achieved substantial and clinically meaningful weight loss of −12.8% (10 mg) and −14.7% (15 mg), respectively, when compared with placebo	Garvey et al.,[172] NCT04657003
	SURMOUNT-3 (phase 3)	579 obese or overweight subjects (2 years/25 visits)	examined if tirzepatide (10 and 15 mg) helps people maintain or improve the weight loss achieved with intensive lifestyle interventions; at 72 weeks, tirzepatide achieved −18.4% weight loss compared with placebo	Wadden et al.,[173] NCT04657016
	SURMOUNT-4 (phase 3)	783 obese or overweight subjects (2 years/25 visits)	study to examine how tirzepatide maintains body weight loss; two study phases: (1) lead-in-phase (all subjects take tirzepatide), (2) treatment-phase (at 88 weeks), participants will either continue tirzepatide or switch to placebo	study completed, NCT04660643
	SURMOUNT-J and CN (phase 3)	261 and 210 obesity subjects (72 and 52 weeks)	obese subjects from a Japanese (SURMOUNT-J) or a Chinese (SURMOUNT-CN) population will be treated with one of two doses of tirzepatide or placebo	study ongoing (NCT04844918); study completed (NCT05024032)
	SUMMIT HFpEF (phase 3)	700 in obese subjects with HFpEF (52 weeks)	efficacy/safety of tirzepatide (vs. placebo) in obese subjects with heart failure with preserved ejection fraction (HFpEF); outcomes: mortality, heart failure events, and exercise capacity	study ongoing, NCT04847557
	TREASURE-CKD (phase 2)	140 in obese chronic kidney disease (CKD) subjects ± T2D (62 weeks)	study using MRI to assess whether tirzepatide (vs. placebo) treatment improves renal function, with focus on kidney hypoxia in relation to fatty kidney disease	study ongoing, NCT05536804

supplemental GLP-1R/GIPR co-agonism could greatly outperform GLP-1R mono-agonism to achieve greater weight loss and elicit further improvements in glucose homeostasis.[85] Subsequent studies entailed the generation of a fatty-acylated version of the peptide with balanced potent activity selective to GLP-1R and GIPR.[157,158] This unimolecular peptide, termed MAR709, harbored several amino acid substitutions, an acylation at a C-terminal lysine, with a saturated C16 palmitic acid. The peptide was resistant to DPP-4 proteolysis and displayed a pharmacokinetic profile suitable for daily clinical use.[157–159] Testifying pharmacologically to the vital contribution of GIPR agonism in the metabolic action of the co-agonist, treatment of diet-induced obese mice with MAR709 produced greater weight loss and further suppression in food intake relative to treatment with the pharmacokinetically matched GLP-1 backbone; this superiority vanished in mice carrying a deletion of *Gipr* in the CNS,[82] or more specifically in GABAergic neurons.[83] These studies essentially identified the brain GIP system as a new regulator of energy metabolism. Taken together, these pioneering preclinical studies established GLP-1R/GIPR co-agonism as an encouraging pharmacological strategy to promote weight loss beyond what can be achieved with GLP-1R agonism alone, thus sealing the fate of gut-based multi-receptor therapies on a fruitful path for future polyagonist discoveries. MAR709 successfully completed phase 1 clinical trials with good tolerability,[85,157,158] concomitant with a meaningful reduction in body weight and HbA_{1c} levels in subjects with T2D.[85,158] However, after completion of phase 2b trials, in which a single tested dose displayed only moderate superiority on body weight and glucose control over liraglutide after 12 weeks of treatment in subjects with T2D,[159] the clinical development of MAR709 was discontinued in favor of proceeding with the clinical advancement of the GLP-1R agonist semaglutide.

Generation of the second GLP-1R/GIPR co-agonist: Tirzepatide

The second important GLP-1R/GIPR co-agonist generated was LY3298176, also termed tirzepatide.[84] This peptide reflects the progression in the chemical optimization of GLP-1R agonists with the application of a fatty-acylated diacid. This modification had prompted semaglutide to be a much longer-acting GLP-1R agonist of greater potency and efficacy than liraglutide. In an analogous fashion, tirzepatide represents a similar evolution in the co-agonist structure of MAR709 by employing a fatty diacid analogous to semaglutide at the same location in a GIP-based peptide that has been modified to include GLP-1 activity.[84] The clinical half-life of tirzepatide is approximately 5 days (~116.7 h), thus permitting OW dosing. A critical difference between tirzepatide and MAR709 is that MAR709 displays "balanced" activity at both GLP-1R and GIPR,[85,157] whereas tirzepatide favors human GIPR over GLP-1R in a ratio of 5:1.[84] Notably, MAR709 and tirzepatide harbor important species-specific differences, with both molecules being fully active agonists at the human GIP receptor; however, only MAR709 being fully active at the mouse GIP receptor. As a result, while MAR709 requires functional GIPR signaling in the CNS to outperform GLP-1R agonism to further reduce body weight and food intake,[82,83] tirzepatide does not lower body weight in *Glp1r*-deficient mice.[154] Moreover, while tirzepatide promotes insulin secretion in murine islets exclusively via the GLP-1R, the peptide stimulates insulin secretion in human islets predominantly via the GIPR.[174] However, consistent with unleashing its full activating potential at the human GIPR,[84,174] *in vitro* studies in human HEK293 cells revealed that tirzepatide activates both GIPR and GLP-1R signaling, and in isolated human pancreatic β cells, the tirzepatide-induced insulin secretion was greater, relative to treatment with either GIP or GLP-1 alone.[84] *In vivo* studies demonstrated that in diet-induced obese mice, tirzepatide enhances glucose-dependent insulin secretion, improves glucose tolerance, stimulates appetite suppression, and promotes remarkable weight loss, the latter a result of increased tissue lipid oxidation[84] (Figure 2). More specifically, tirzepatide treatment was reported to drive a tissue-specific increase in glucose disposal, preferentially into epididymal white adipose tissue, brown adipose tissue (BAT), and skeletal muscle,[154] an effect associated with transcriptional upregulation in genes associated with branched-chain amino acid catabolism in BAT.[156,160] The peptide was further shown to induce a switch of macronutrient intake by selectively dampening palatable high-fat/sweet-taste preference to a low-fat chow diet preference.[175] On a similar note, studies in musk shrews and mice revealed that GIPR mono-agonist administration attenuated the emetic effect of GLP-1R agonism,[81,176] a highly desirable property that may contribute to increased tolerability of the GIP-based drugs relative to GLP-1-based monotherapies at higher doses.

Clinical findings of tirzepatide

Tirzepatide was approved by the FDA for the treatment of T2D in May 2022, then later for the treatment of obesity in November 2023 (Figure 3). The peptide has been extensively evaluated in numerous clinical trials, many of which are still ongoing (Table 1). The multi-center SURPASS 1–6 trials evaluated the efficacy of tirzepatide (5, 10, or 15 mg OW) to treat T2D in Hispanic and non-Hispanic White subjects with obesity. SURPASS-1 for instance, assessed the efficacy of tirzepatide relative to placebo in subjects with obesity and T2D, which were recruited at 52 hospitals in India, Japan, Mexico, and the US. Following 40 weeks of treatment, ~92% of subjects receiving tirzepatide achieved an HbA_{1c} of <7.0% relative to 19% receiving placebo, with 52% vs. 1% of subjects reaching an HbA_{1c} of <5.7%.[161] Similar glycemic benefits were observed in subsequent SURPASS trials, whereby tirzepatide proved superior in improving glycemic control relative to treatment with semaglutide (1 mg),[162] insulin degludec,[163] insulin glargine,[165,166] and insulin lispro,[167] importantly, with preserved efficacy and without compromising safety in subjects at risk for cardiovascular diseases.[165] The SURPASS J-mono trial evaluated the glycemic effects of tirzepatide in Japanese subjects with T2D, demonstrating that after 52 weeks of treatment, HbA_{1c} levels were lowered by −2.8%, relative to −1.3% in subjects receiving dulaglutide.[168] Similar impressive improvements in glycemia were reported from the SURPASS J-combo trial, in which tirzepatide was administered for 52 weeks as add-on therapy to sulfonylureas, biguanides, α-glucosidase inhibitors, thiazolidinediones, glinides, or sodium-glucose transport protein 2 (SGLT2) inhibitors in Japanese subjects with poorly controlled T2D.[169] Likewise, the SURPASS AP-combo trial examined the effects of tirzepatide in comparison with insulin glargine in T2D subjects originating from China,

South Korea, Australia, and India.[170] Taken together, multiple SURPASS trials are still ongoing that are likely to achieve similar positive metabolic outcomes, including (1) the SURPASS-CVOT trial in subjects with T2D that have a history of cardiovascular disease,[177] (2) the SURPASS-EARLY study in subjects with T2D that were diagnosed no more than 4 years before enrollment, (3) the SURPASS-SWITCH trial that evaluates the glycemic effects of subjects that were switched from dulaglutide to tirzepatide, and (4) the SURPASS-PEDS study that examines the metabolic effects of tirzepatide treatment in children with T2D.

To evaluate the efficacy of tirzepatide treatment primarily in the context of obesity, the multi-center SURMOUNT trials were launched (Table 1). In the SURMOUNT-1 trial for obese subjects without T2D, 72 weeks of tirzepatide treatment achieved weight loss of up to −20.9%, relative to placebo controls.[178] Similarly, in a study with comparable treatment duration, albeit in subjects with obesity and T2D, tirzepatide reduced body weight by −14.7%, relative to −3.2% in placebo controls.[172] Despite the slight differences based on the subject cohort and the duration of treatment, this magnitude of weight loss was largely consistent with the SURPASS trials.[161–163,165–170] In the SURMOUNT-3 trial, the effects of tirzepatide were assessed in obese subjects without T2D that underwent intensive lifestyle modifications; the supplemental therapeutic treatment with tirzepatide for 72 weeks reduced body weight by −18.4%, relative to a 2.5% weight gain evident in placebo controls.[173] Ongoing SURMOUNT trials include the SURMOUNT-MMO, in which subjects will be monitored for 5 years for any appearance of cardiovascular adverse events, in addition to the SURMOUNT-OSA trial, in which the effects of tirzepatide on obstructive sleep apnea will be assessed. Lastly, either ongoing or in the pipeline are the SUMMIT 1–6 trials, which aim to evaluate the action of tirzepatide for the treatment of obesity and its associated diseases.[171] For instance, the SUMMIT-HFpEF (heart failure with preserved ejection fraction) trial will examine obese subjects with heart failure, as well as the TREASURE-CKD study that will address obesity in the context of chronic kidney disease.

The global scientific interest in tirzepatide is increasing at an exponential rate, and as such, several other clinical trials beyond the scope of this review are currently underway. Table 1 provides a comprehensive summary of the completed and ongoing trials for the two GLP-1R/GIPR co-agonists, MAR709 and tirzepatide, which primarily focus on obesity and T2D, with endpoints of HbA_{1c} levels, glycemic efficacy, weight loss, and lipid profiles. Combined, the SURPASS and SURMOUNT trials have verified the safety of tirzepatide to be consistent with GLP-1R agonists, along with its enhanced efficacy at maximal doses of 5, 10, and 15 mg (Table 1).[161–166,171–173] Additional noteworthy studies regarding tirzepatide include phase 1 trials utilizing magnetic resonance imaging to assess the central regions of the brain that regulate food intake and energy expenditure (NCT04311411, NCT04081337), along with a study to examine gastric emptying in obese or T2D subjects (NCT04407234). Recently, tirzepatide treatment was also shown to improve insulin sensitivity, enhance pancreatic β cell function, and slow glucose excursions during meal tolerance tests in T2D subjects.[179] Of note, the body weight-lowering efficacy of tirzepatide and semaglutide was recently compared in a meta-analysis study composed of data from over 41,000 individuals. After 1 year of treatment, weight loss of ≥10% was achieved in 62.1% of subjects receiving tirzepatide and in 38.0% of subjects receiving semaglutide.[180] Preliminary evidence further suggested that treatment with tirzepatide led to additional weight loss of −4.3% after 6 months and −7.2% after 12 months, without differences in the occurrence of gastrointestinal adverse effects.[180] Collectively, it is apparent that GLP-1R/GIPR co-agonists have firmly established an unprecedented benchmark for medicinal drugs aiming to treat T2D and obesity.

The clinical experience with tirzepatide has thus captured tremendous attention and fueled much interest in the development of other combinations of multi-receptor activating peptides. Several questions remain that are centered on the definitive mechanism by which GLP-1R/GIPR co-agonists achieve their remarkable metabolic outcomes. GIPR agonism has been preclinically characterized to amplify the glycemic and weight-lowering performance of GLP-1R/GIPR co-agonism, when compared with selective GLP-1R agonism,[82,83] and, as such, should be credited as an essential component that helps achieve the optimal metabolic benefits of the co-agonist. Indeed, with some clinical trials scheduled to compare GIP with semaglutide for T2D (NCT05078255) and glucose tolerance in individuals with genetically altered GIPR, GLP-1R, and GLP-2R function (NCT06194955), it is highly likely that in the pipeline, clinical studies utilizing GIPR mono-receptor agonists will report meaningful metabolic benefits in relation to T2D and obesity. It is also equally plausible, albeit yet to be clinically validated, that GIPR agonists will prove successful in combination therapies with other non-incretin-based drugs. However, the specific question of whether agonism requires molecular integration into a single molecule or can be utilized in an adjustable ratio is yet to be fully addressed. Other unimolecular co-agonists in clinical development include an oral formulation of a GLP-1R/GIPR co-agonist peptide, sponsored by Novo Nordisk, which is in phase 1 clinical trials for T1D subjects. Similarly, fixed-dose combination (FDC) Sema-OW GIP, is a subcutaneous injectable combination of semaglutide with a novel GIPR agonist, termed NNC0480-0389. This drug combination is currently under study in healthy subjects, in addition to obese and T2D individuals (NCT04259801), and is projected to enter phase 2 trials in the near future. Taken together, it is now evident that by partnering GLP-1R and GIPR to generate incretin-based co-agonists achieves spectacular metabolic outcomes with enhanced therapeutic dosing. The progression in the performance of these GLP-1R/GIPR co-agonists follows the chemical trend first developed in GLP-1R agonism with liraglutide and semaglutide to now achieve a much greater metabolic sequel.[84,85]

Taken together, two GLP-1R/GIPR peptides shone in the bench-to-clinical spotlight of sophisticated unimolecular co-agonists, and as such, great credit should be given to these initial developments that created a solid foundation for the much-anticipated multi-receptor triagonist peptides to follow. Finally, while the precise mechanisms underlying the coordinated benefits of GLP-1R/GIPR co-agonism are yet to be fully unraveled, it is conceivable that adipose tissue as a peripheral target may play a significant role in the regulation of energy balance, given the sizable degree of weight reduction and loss in fat mass. As

such, future preclinical studies regarding the mechanistic basis of co-agonist peptide action should prove illuminating.

GLP-1R/GIPR/GCGR triagonists

Based on the preclinical success of the GLP-1R/GCGR co-agonists[112] and GLP-1R/GIPR co-agonists,[85] it was naturally intuitive to assume that a single peptide that displays balanced activity at all three target receptors would further enhance glycemic control and accelerate weight loss, with the hope of surpassing what had already been achieved with bariatric surgery.[8,109] The chemical challenge, however, was to satisfy the structural requirements for agonism at three related but different receptors, where the native hormones GLP-1, GIP, and glucagon are highly specific in their interactions. Appreciably, the pharmacology of the first generation of such unimolecular triagonists already proved superior to any best-in-class single or co-agonist peptides at that time[181–185] (Figure 2).

Preclinical findings that launched unimolecular triagonism

Informative preclinical studies that began much before the achievement of weight loss in humans, which now exceeds 20% with the best-in-class co-agonists, described the synthesis and characterization of several unimolecular GLP-1R/GIPR/GCGR triagonists.[181–184,186] These preclinical findings elegantly set the stage for the ongoing clinical trials in obesity and T2D using triagonist peptides. The first preclinically established unimolecular GLP-1R/GIPR/GCGR triagonist was MAR423. This peptide was shielded from DPP-4 recognition through an aminoisobuturic acid at position 2, while the lysine at position 10 was fatty-acylated with a palmitic acid through a γ-glutamic acid linker.[181] Distinct amino acid substitutions were introduced into the center of the peptide to restore balanced glucagon receptor activity, while the C-terminal end of exendin-4 was attached to display balanced full agonism at all three receptors.[181] In diet-induced obese mice, MAR423 displayed impressive dose-dependent body weight-lowering effects of 26.6% in 20 days, compared with 15.7% with GLP-1R/GIPR co-agonist treatment. The peptide further reduced food intake and fat mass, enhanced glycemic control, reduced hypercholesterolemia, and improved hepatic lipid metabolism, relative to GLP-1R agonism or balanced GLP-1R/GIPR co-agonism[181] (Figure 2). Mice individually lacking each of the three receptors, or pharmacological antagonism of each receptor, confirmed the functional relevance of each of the three peptide entities,[181] which was further apparent with glucagon induction of energy expenditure and lipid utilization.[105,181] MAR423 further improved dyslipidemia and ameliorated hepatic steatosis in obese mice, notably even at doses where the drug only had marginal effects on body weight and satiety.[183]

Following the publication of the first triagonist, a series of similar peptides emerged, but with notable differences in duration of action and activity at each target receptor.[182,184,186] Following 2 weeks of treatment, such a biochemically refined second-generation triagonist exhibited impressive weight loss of >30% in diet-induced obese rodents, with superior weight loss relative to the best-in-class GLP-1R/GIPR co-agonists.[182] This GLP-1R/GIPR/GCGR triagonist, LY3437943, is based on the GIP sequence, in which amino acid substitutions were stepwise introduced to achieve triple agonism.[84,186] The 39 amino acid peptide carries non-natural amino acids at positions 2, 13, and 20, which not only protect from DPP-4-mediated degradation but also enhance the activity at the receptors for GIP and glucagon.[186] A C20 fatty diacid was further anchored onto the lysine at position 17 to enhance bioavailability through albumin binding.[186] In contrast to the balanced triagonist MAR423,[181,182] LY3437943 displayed balanced activity for GCGR and GLP-1R but enhanced potency at the GIPR. In diet-induced obese mice, LY3437943 very effectively lowered body weight by ∼45%, which was largely preserved at thermoneutrality.[186] Weight loss was further accompanied by a reduction in fat mass, a transient suppression in food intake, along with marked improvements in glycemic control.[186] Notably, the LY3437943 triagonist peptide outperformed the GLP-1R/GIPR co-agonist tirzepatide to achieve a greater degree of weight loss,[84] an observation attributed to an increase in energy expenditure, which remarkably accounted for ∼30%–35% of the weight lost in diet-induced obese mice and was diminished upon antibody-based inhibition of the GCGR.[186]

SAR441255 is a synthetically balanced unimolecular GLP-1R/GIPR/GCGR triagonist, structurally based on the exendin-4 sequence with selected substitutions and palmitic acid acylation.[184] In lean cynomolgus non-human primates, positron emission tomography imaging revealed higher receptor occupancy to GLP-1R and GCGR.[184] In diet-induced obese mice, SAR441255 was shown to alleviate hyperglycemia and reduce body weight by ∼14.1%; moreover, when compared with a GLP-1R/GCGR co-agonist, this effect was primarily ascribed to an increase in energy expenditure.[184] Similar superior metabolic outcomes were also observed in obese diabetic cynomolgus non-human primates.[184]

HM15211 (also termed ^{LAPS}triple agonist) is a long-acting GLP-1R/GIPR/GCGR triagonist peptide based on the glucagon sequence and conjugated to a human glycosylate crystallizable fragment.[187] HM15211 was examined in rodent and cynomolgus non-human primate models of obesity, T2D, and MASLD.[187] This triagonist outperformed liraglutide in terms of weight loss and further improved hyperglycemia, increased energy expenditure, lowered hypercholesterolemia, and ameliorated hepatic steatosis and fibrosis.[187] The latter metabolic benefits were attributed to the peptide's anti-inflammatory properties and its ability to reduce transforming growth factor β production.[185,187] Consistent with these findings, a triple separate physical mixture of GLP-1R, GIPR, and GIPR mono-agonists also served to dampen hepatic triglyceride accumulation in a mouse model of MASLD and fibrosis.[188] These results thus highlight the tremendous potential for triagonist peptides to treat obesity-associated MASLD. Other less-characterized triagonists include YAG/glucagon,[189] [D-Ala^2]GLP-1/glucagon,[190] and [D-Ala^2]GIP/Oxm.[191]

Collectively, preclinical studies identified that unimolecular triagonist peptides are vastly superior to existing co-agonists and mono-agonists in the regulation of body weight, satiety, hepatic lipid metabolism, and glycemic control (Figure 2). The unique contribution of each hormone allows for enhanced synchronized metabolic outcomes. The current preclinical mechanistic logic in how each receptor activity contributes to weight loss could be as

follows: (1) GLP-1R agonism primarily serves to reduce food intake and improve glycemic control,[192,193] (2) GCGR agonism stimulates in increase in energy expenditure,[182,186] and (3) GIPR agonism may serve as a metabolic booster to potentiate the satiety effects of GLP-1, improve insulin sensitivity, and buffer the hyperglycemic liability of glucagon to permit more aggressive GCGR agonism.[181] The basic cellular biology of GIPR is likely the most complex and puzzling of the three receptors. At this stage, we cannot exclude the specific actions of the GIPR in the periphery, particularly in adipose tissue, where we need to gain a better understanding of what the receptor is capable of achieving upon activation in white fat.

Clinical findings in the development of triagonist peptides

In a clinical setting, the GLP-1R/GIPR/GCGR triagonist peptides that progressed to clinical development included MAR423,[181–183] LY3437943 (retatrutide),[186] SAR441225,[184] and HM15211 (Table 2; Figure 3). The first to advance to clinical study was MAR423 (also referred to as NN9423), which, however, based on its necessity for once daily administration, has been abandoned in favor of an OW version.[182]

A single dose of another triagonist peptide, retatrutide, was shown to elicit impressive weight loss of up to −3.52 kg with a 6 mg dose, which persists for up to 6 weeks.[186] Comparable weight loss with tirzepatide was achieved after four weekly doses.[186] Interestingly, healthy subjects exhibited a transient suppression in appetite, a reduction in endogenous glucagon levels, an increase in systemic β-hydroxybutyrate levels, along with an improved lipid profile.[186] In a phase 1 proof-of-concept study in T2D subjects, the efficacy of OW ascending doses of retatrutide (0.5–12 mg), compared with dulaglutide (1.5 mg), or placebo (NCT04143802)[194] was explored. Following 12 weeks of treatment, retatrutide reduced absolute HbA_{1c} levels by −1.90%, compared with −0.96% and −0.34% with dulaglutide or placebo, respectively.[194] Retatrutide further induced dose-dependent weight loss of −8.65 kg from baseline at the highest dose[194] (Table 2). In these studies, retatrutide displayed a safety profile not overtly different to selective GLP-1R agonism, with some nausea being the most frequently reported adverse effect.[186,194] The triagonist further lowered systolic and diastolic blood pressure, with a subtle transient increase in heart rate; the latter consistent with GLP-1R-based therapeutics.[186,194] Combined, these initial clinical studies indicated that retatrutide has vast potential for differential performance, relative to semaglutide and tirzepatide, and the peptide currently constitutes the leading edge in incretin-based obesity therapy.

More recently, in a phase 2 trial, retatrutide achieved a stunning record of ∼24.2% placebo-corrected weight loss in obese subjects without T2D after 48 weeks of treatment[196] (Table 2). It is the current benchmark for the greatest weight loss ever reported with this class of anti-obesity drug candidates. In comparison, placebo-corrected weight loss induced by tirzepatide or semaglutide at this treatment time point in a comparable study population was ∼17%[178] and ∼13%,[197] respectively. In a back-to-back published phase 2 study composed of subjects with obesity and T2D, 36 weeks of retatrutide treatment reduced body weight by ∼16.9%, compared with ∼2% or ∼3% with dulaglutide or placebo, respectively; furthermore, the triagonist lowered HbA_{1c} levels by ∼2%, compared with ∼1.4% or ∼0.01% with dulaglutide or placebo, respectively, at the 24-week time point.[195] Finally, in a phase 2 sub-study in subjects with MASLD, retatrutide significantly decreased liver fat, displaying a substantial reduction of up to 86% over a 48-week period[198] (Table 2), which suggested that the peptide has the potential to resolve MASLD. Taken together, these clinical studies highlight the powerful impact that retatrutide has on glucose and lipid metabolism, with an unprecedented level of weight loss that appears to be continuing at the study end.[196]

The third long-acting unimolecular GLP-1R/GIPR/GCGR triagonist in clinical development is HM15211 (Figure 3). In a phase 1 study, the addition of GIPR activity to GLP-1R/GCGR co-agonism was reported to greatly potentiate the weight-lowering and glycemic efficacy in overweight subjects (NCT04521738).[184] In a subsequent phase 1 trial, multiple ascending doses of HM15211 will be utilized in 66 obese subjects with MASLD (NCT03744182). Similarly, in an ongoing phase 2 study, 217 subjects with biopsy-confirmed MASH will be assessed (NCT04505436) (Table 2). The latter clinical trial is an important benchmark investigation, as HM15211 specifically displays much potential in obesity-related liver disease, as a point of distinction for the more customary focus on glucose control and body-weight-lowering capabilities.

Remaining questions surrounding unimolecular triagonism

The medicinal objectives of the unimolecular triagonists were to harness three complementary signaling mechanisms in weight reduction to achieve unprecedented efficacy, which could rival or even surpass bariatric surgery. One question in particular, however, is how much of the three receptor activities exhibit independent or overlapping mechanisms in weight loss?

Initially, the spectacular weight loss achieved with GLP-1R/GIPR co-agonism begged the question of how best to integrate GCGR agonism. The biological impasse that glucagon can induce hyperglycemia is an immediate and obvious brake to the degree of GCGR agonism. Additionally, glucagon has vascular effects that must be successfully managed or risk the full improvement in cardiovascular outcomes achieved with less aggressive forms of therapy. Nonetheless, the deepened appreciation for the role that glucagon has on energy expenditure has placed this hormone in a new light and rekindled its consideration as a component that could provide additional weight loss when needed or to sustain the weight loss that may otherwise wane through some form of supplementation.[103] A recent report also puts forth potent reno-protective effects that are exerted through the GCGR in the kidney.[108] The clinical results regarding tirzepatide appear to anchor GIPR activity as much as GLP-1 activity in the treatment of obesity. However, questions remain as to whether the GIPR is predominantly a metabolic booster to GLP-1 or, with sustained efficacy, increasingly emerging as a primary contributor to maintaining metabolic health. The importance of GIP in triple agonism may be doubly so, where these peptides appear to tolerate glucagon activity much more than GLP-1R/GCGR co-agonism. The role of GIPR activity within the triagonist may prove more complex, and albeit speculative at this stage, its function could include the stimulation of energy-wasting futile cycling pathways. Future genetic studies will no doubt shine light on the shadow of GIP function.

Table 2. Completed and ongoing clinical trials of the unimolecular GLP-1R/GIPR/GCGR triagonists

Molecule and company	Development phase and status	Indication and duration	primary outcomes	References and Clinical Trial.gov ID
MAR423 (NN9423, NNC9204-1706); Novo Nordisk	phase 1	60 overweight or obese subjects (16 weeks)	ascending-dose study to assess the safety and efficacy of NNC9204-1706 in healthy or obese subjects, compared with placebo	studies completed; NCT03095807; NCT03661879
Retatrutide (LY3437943, "Triple G"); Eli Lilly	phase 1	45 healthy subjects (71 days)	first-in-human single ascending-dose study of retatrutide (0.1–6 mg), relative to placebo; weight loss of −3.52 kg (6 mg retatrutide) persisted up to day 43 after a single dose; pharmacokinetics (PKs) supported once weekly dosing; decrease in systemic fasting glucagon, triglyceride levels, with an increase in β-hydroxybutyrate	Coskun et al.,[186] NCT03841630
	phase 1	72 T2D subjects (12 weeks)	proof-of-concept study: after 12 weeks, once weekly retatrutide treatment (0.5–12 mg) reduced placebo-adjusted HbA_{1c} levels up to −1.60% at the 3/6 mg dose; weight loss achieved was −8.96 kg with the highest dose	Urva et al.,[194] NCT04143802
	phase 2	281 T2D subjects ± metformin (24 weeks)	first published clinical trial to primarily evaluate the multiple ascending doses of retatrutide (0.5–12 mg), compared with dulaglutide and placebo, in T2D subjects; mean changes in HbA_{1c} achieved for retatrutide ranged from −0.43% (0.5 mg) to −2.02% (12 mg), vs. −1.41% for dulaglutide (1.5 mg)	Rosenstock et al.,[195] NCT04867785
	phase 2	338 adults with obesity (48 weeks)	first published clinical trial on retatrutide in the context of obesity; retatrutide (1–12 mg) elicited the highest weight loss recorded for an anti-diabetic/ obesity medication to date; retatrutide (12 mg) achieved −24.2% weight loss, compared with placebo; in obese female subjects, massive −28.5% weight loss was recorded	Jastreboff et al.,[196] NCT04881760
	phase 1	64 T2D and 32 obese subjects (12 and 20 weeks)	multiple ascending-dose study assessing the safety, PK, and pharmacodynamics (PC) of retatrutide, when given to Japanese patients with T2D, or overweight/obese Chinese subjects, vs. placebo	study completed (NCT04823208); study ongoing (NCT05548231)
	phase 3 (TRIUMPH-3)	1,800 obese subjects with CVD (113 weeks)	examine the efficacy and safety of retatrutide once weekly in participants with obesity CVD, compared with placebo	study ongoing NCT05882045
	phase 1	20 subjects with renal impairment (5 weeks)	assess the PK (the duration it takes retatrutide to reach the bloodstream, then be excreted) in subjects with renal impairment, in comparison with healthy subjects	study ongoing NCT05611957
HM15211 (^{LAPS}triple agonist, formerly SAR441255) (Sanofi, then Hanmi Pharmaceuticals)	phase 1	48 healthy lean-to-overweight subjects (8 weeks)	study showing that a single dose of SAR441255 (3–150 μg) treatment had potent body-weight-lowering effects and improved glycemic control, after a meal tolerance test, a reduction in glucose, insulin, and C-peptide levels was evident	Bossart et al.,[184] NCT04521738
	phase 1	40 obese subjects (4 weeks)	first-in-human clinical trial was completed using a single ascending-dose of HM15211 vs. placebo, which confirmed the safety and tolerability in obese subjects	study completed NCT03374241
	phase 1	66 obese subjects with MASLD (12 weeks)	study to evaluate the safety, tolerability, PK, and PC of multiple ascending doses of HM15211 vs. placebo in obese subjects with MASLD	results submitted NCT03744182
	phase 2	217 subjects with biopsy-confirmed MASH (12 months)	study to confirm the efficacy, safety, and tolerability of HM15211 treatment, vs. placebo, in subjects with biopsy-confirmed MASH	study ongoing NCT04505436

But for now, the broader history of anti-obesity drug development instructs that we progress with due caution, as there have been other forms of pharmacology that have unsafely promoted weight loss. The task before us is to use these pharmacological tools and preclinical mechanistic observations in the most intelligent and informed manner in treatment of obese and T2D individuals.

POTENTIAL SIDE EFFECTS OF MONO- AND MULTI-RECEPTOR AGONISTS

An important aspect to consider is that there is a broad consensus in the therapeutic field that incretin-based interventions cannot reset the "lipostat," i.e., upon stopping drug treatment, both in rodent models and in clinical studies, body weight regain is frequently observed, as the weight lost ultimately reverts back to baseline levels.[199] In other words, there is a high rate of "recidivism" similar to what is observed in lifestyle intervention trials. Indeed, discontinuation of either semaglutide[199,200] or tirzepatide[201] leads to a significant rebound in body weight gain. This should, however, not be an entirely unexpected response, given that similar effects are observed following treatment withdrawal from cardiometabolic disease therapeutics, i.e., for hypertension or hypercholesterolemia. While this notion is generally true, there is recent evidence to suggest that some degree of the initial weight loss may be retained following a wash-out period of the drug. In the SURMOUNT-4 trial, obese subjects that were treated with tirzepatide for 36 weeks exhibited a reduction in body weight of ~21%.[201] Individuals that received tirzepatide for an additional 52 weeks lost an additional 6%, whereas subjects that received placebo for the 52-week follow-up period regained 14% of their body weight.[201] Despite this weight regain, the subjects that were administered placebo still maintained an ~10% weight loss compared with their starting body weight, which is highly meaningful. Albeit, at this stage, it is not known whether body weights will fully revert to baseline levels over a more prolonged follow-up period. Taken together, this is a sober realization that novel weight loss pharmacotherapies, despite their unprecedented effectiveness, may not be regarded as the ultimate cure for obesity. Nevertheless, at present, this suggests that to reap the full benefits of weight loss, lifelong drug exposure may be required. This prospect does, however, set the challenge for next-generation pharmacotherapy that will hopefully offer a permanent treatment solution for obesity, even after treatment discontinuation.

Another concern is that the degree of weight loss induced by drug treatment is not exclusively due to a loss in fat mass; rather, there is a significant amount of muscle loss that may accompany this. An ongoing debate is whether this loss in lean mass is simply a reflection that a reduction in body weight requires less muscle mass to provide overall mechanical support or whether the loss in lean mass poses a significant health concern to the individual. This can be a considerable issue to individuals who repeatedly cycle on and off drug treatment, resulting in a "yoyo body weight" phenomenon, with each cycle associated with an increase in fat mass, along with a reduction in muscle mass. This aspect is more of a concern in older patient populations that suffer from age-related sarcopenia, even prior to mono- or multi-receptor agonist exposure. However, these issues could potentially be dampened by a series of countermeasures, such as inhibition of the activin type II receptor axis or activation of the apelin receptor pathway. Along the same lines, the relative performance of mono- or multi-receptor agonists in sub-populations of T2D, for instance, lean T2D subjects, remains unknown. Lean T2D is a distinct clinical entity, and given the >20% weight loss achievable in obese T2D subjects, it remains to be determined whether lower doses of drug treatment can maintain glycemic control while minimizing unwarranted weight loss, as this could potentially be associated with a reduction in lean mass in otherwise lean T2D subjects.

Finally, we should mention the logistical and socioeconomic issues associated with the widespread use of these novel drug interventions. At present, supply issues still prevail, with demand vastly exceeding supply. Unquestionably, time and a more competitive marketplace will resolve these issues. However, what will continue to be an issue is the high monthly costs that individuals must bear in light of the fact that the majority of health insurance plans do not cover the costs of anti-obesity medications. The major concern is that this leads to further health disparities, with patients who need these interventions the most finding themselves unable to afford them or gain access to them.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

The progress witnessed in the last decade in the management of obesity and its related diseases has been nothing short of stunning. Advances in metabolism over the last century were accelerated in the last few decades by collective biotechnologies to fulfill the incretin hypothesis. GLP-1 proved exceedingly effective in the management of T2D-associated hyperglycemia, without the risk of hypoglycemia commonly associated with insulin and sulfonylureas. Through state-of-the-art iterative enhancements in its pharmaceutical properties, highly effective selective GLP-1R peptide agonists emerged for the treatment of T2D. The therapeutic focus then transitioned to obesity, as preclinical and clinical observations revealed body weight lowering as an adjunctive benefit in the management of T2D-associated obesity. The first forms of therapy proved comparably effective to the conventional non-peptide forms in providing a mid-single-digit lowering in body weight, with reductions in adverse cardiovascular events. The serendipitous step forward emerged when semaglutide, a peptide designed for increased patient convenience in reducing injectable dosing from daily to OW, proved doubly efficacious in lowering body weight to beyond 10%, when compared with a daily dosing of the chemically related peptide, liraglutide. By clinically exceeding the anti-obesity efficacy of liraglutide, semaglutide transformed the vision of what was pharmaceutically possible and thus launched the medicinal management of obesity, despite reaching only a fraction of what can be achieved through bariatric surgery. Shortly thereafter, the clinical performance of semaglutide in the treatment of T2D and/or obesity was substantially eclipsed by the integrated pharmacology of GIP and GLP-1 in tirzepatide. The peptide served to double the conviction for the prospect of body weight-lowering efficacy comparable to bariatric surgery, a goal that was within reach, for the first time, through the use

of multi-mechanism pharmacology. While the clinical results validated this prospect, the short time interval in moving from semaglutide to tirzepatide is a manifestation that the seeds of invention had been planted more than a decade earlier, with preclinical observations that predicted this outcome, and an even greater performance with further iterations in mechanisms of action. With respect to GIPR agonism, while a definitive mechanism in how GIPR signaling impacts energy balance is yet to be reported, GIPR activation has been associated with improved adipose tissue health.[78,139,156,202] Considering the prominent role of adipose tissue in energy homeostasis,[92,203] it is plausible that GIPR agonism in fat could harness beneficial effects on metabolic homeostasis.

The advance beyond selective GLP-1 agonism was also rooted in controversy. Beginning with glucagon agonism, which is counterintuitive to glucagon's diabetogenic pharmacology, with decades of work in the literature that unsuccessfully pursued its antagonism for the treatment of diabetes. The deeper appreciation that glucagon, much like insulin, has a larger scope of pharmacology than just acute glucose management, coupled with the integration with GLP-1 and GIP, has enlightened the prospect of utilizing the hormone for constructive purposes. Nonetheless, it needs to be approached with great caution, particularly relating to its potential cardiovascular effects, which are more challenging to assess and potentially more difficult to reverse than its impact on glycemic control. The success with GLP-1 and the similar emerging success with GIP should not lessen our concern in the management of glucagon, as the former are physiological incretins and more alike than the latter hormone. The integration of glucagon and GIP agonism into GLP-1 pharmacology represents the most advanced form of multimode therapy that is achieving extraordinary progress, currently advancing to the last stage of clinical development. Figure 3 highlights some of the next-generation therapeutics for the treatment of obesity and T2D, with the relative GLP-1, glucagon, and GIP gut-hormone contributions in each agonist peptide and the remarkable weight loss achieved for each.

The ongoing observations that GIP agonism and antagonism can achieve similar preclinical outcomes in lowering body weight are perplexing. The physiological role of GIP in other endocrine functions, such as the maintenance of bone health, is one of appreciable importance.[204] For instance, GIPR agonism within multi-receptor agonists improves bone metabolism.[184] As such, the unintended prospect that inhibiting GIP activity in a T2D patient population with reduced bone mineral density could represent a chronic adverse risk that needs to be addressed. A recent study utilizing a GIPR-blocking antibody conjugated with a potent GLP-1R agonist in obese subjects has reignited interest in such multimode therapy.[205] The sustained body weight-lowering efficacy at monthly doses that are more than a 100-fold higher than that commonly employed with selective GLP-1 agonists, without gastrointestinal adverse effects, is difficult to explain. Future studies will no doubt delineate how this apparent discrepancy is possible. In light of this, for the time being, it seems best to adhere to the wisdom that "*the test of a first-rate intelligence is the ability to hold two opposed ideas in the mind at the same time and still retain the ability to function*."

In conclusion, bariatric surgery still stands as the benchmark for weight loss, triggering weight reduction of ~25%–30% within a 1- to 2-year period.[206] Semaglutide has reported weight-lowering capabilities of approximately half this magnitude, with tirzepatide meaningfully reducing the relative remaining difference to surgery in half. Preclinical results and emerging clinical data with triple agonism indicate further advances. There is no doubt that there have been milestone strides achieved in the path to success for multi-receptor agonists as therapeutic agents for the treatment of obesity and diabetes. It is an exciting time as these medicines move to larger populations beyond clinical trials and hopefully stand the test of time in real-life practice. The promise of anti-obesity therapy at this level of efficacy to reduce the burden of T2D and the related cardiovascular diseases, as well as a potential impact on chronic kidney disease, cancer, osteoarthritis, pain management, and other sequelae for which excess body weight is a risk factor, is beginning to be put to the real-world test. Multi-receptor agonist therapeutics are henceforth acknowledged as a hopeful avenue that will undoubtably change the trajectory of the relentless rise in global obesity.

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DECLARATION OF INTERESTS

C.M.K. and P.E.S. have sponsored research agreements with Eli Lilly. M.H.T. was a member of the Research Cluster Advisory Panel (ReCAP) of the Novo Nordisk Foundation between 2017 and 2019. He received funding for his research projects by Novo Nordisk (2016–2020) and Sanofi-Aventis (2012–2019). He consulted twice for Boehringer Ingelheim Pharma GmbH & Co. KG (2020 and 2021) and delivered a scientific lecture for Sanofi-Aventis Deutschland GmbH (2020). As CEO and CSO of Helmholtz Munich, he is co-responsible for numerous collaborations of the employees with a multitude of companies and institutions worldwide. In this capacity, he discusses potential projects with and has signed/signs contracts for the Helmholtz institute(s) related to research collaborations worldwide, including but not limited to pharmaceutical corporations like Boehringer Ingelheim, Novo Nordisk, Roche Diagnostics, Arbormed, Eli Lilly, SCG Cell Therapy, and others. As the CEO and CSO of Helmholtz Munich, he was/is further overall responsible for commercial technology transfer activities. M.H.T. confirms that, to the best of his knowledge, none of the above funding sources or collaborations were involved in or had an influence on the preparation of this manuscript. M.H.T. is a former member of the scientific advisory board of ERX, which is developing celastrol, but has no current competing interests. R.D.D. is the co-inventor of multiple patents pertaining to this field that are owned by Indiana University. He was co-founder of Marcadia Biotech and a former employee at Eli Lilly Research labs and Novo Nordisk, advancing drug candidates associated with the subject of this review. T.D.M. receives research funding from Novo Nordisk; however, these funds are unrelated to the work described here. T.D.M. further

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