OBESITY Reviews
WORLD OBESITY

The incretin/glucagon system as a target for pharmacotherapy of obesity

Journal:	*Obesity Reviews*
Manuscript ID	OBR-06-21-5187.R1
Manuscript Type:	Review
Date Submitted by the Author:	n/a
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Keywords:	Overweight, dual-agonist, GLP-1, glucagon

Summary

Obesity is a chronic, multifactorial, relapsing disease. Despite multicomponent lifestyle interventions, which can include pharmacotherapy, maintaining bodyweight loss is challenging for many people. The pathophysiology of obesity is complex, and the currently approved pharmacotherapies only target a few of the many pathways involved; thus, single-targeting agents have limited efficacy. Proglucagon-derived peptides, glucagon and the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), represent attractive targets for managing obesity and metabolic disorders because they may have direct roles in multiple mechanisms including satiety, energy homeostasis and lipolytic activity. Unimolecular dual and triple agonists targeting glucagon and incretin hormone receptors have been shown to promote bodyweight loss, lower glucose levels and reduce food intake in animal models of obesity. Multiple dual receptor agonists are in clinical development for the treatment of obesity, including GLP-1/GIP and GLP-1/glucagon receptor agonists. The extent to which glucagon contributes to treatment effects remains to be understood, but it may promote bodyweight loss by reducing food intake, while concomitant GLP-1 receptor agonism ensures normal glucose control. Further research is required to fully understand the molecular mechanisms of action and metabolic effects of both dual and triple receptor agonists.

1. INTRODUCTION

The overwhelming increase in the prevalence of obesity and people who are overweight in recent years represents one of the greatest global threats to public health. Worldwide, the prevalence of obesity has tripled since 1975, with over 650 million adults affected in 2016.[1] Obesity is now recognised as a multifactorial disease, characterised by abnormal or excessive fat accumulation that presents a risk to human health.[2] Obesity (a body mass index (BMI) ≥ 30 kg/m^2) and being overweight (a BMI of 25–29.9 kg/m^2)[3] are associated with several health conditions including diabetes, cardiovascular disease, some forms of cancer, musculoskeletal disorders (especially osteoarthritis), sleep apnoea, asthma, gallstones, depression and non-alcoholic steatohepatitis (NASH).[1, 2, 4-7] Obesity is a complex, chronic, relapsing disease: weight gain can be progressive, occurring over many years, and weight loss is difficult to achieve and even more so to maintain.[2, 8, 9] In a meta-analysis of 29 studies, more than half (56%) of lost weight was regained within 2 years and 79% was regained by Year 5.[9] Furthermore, some people with obesity do not consider themselves overweight, while others who do consider themselves overweight have no desire to lose weight.[10] Around one third of people with obesity would like to lose weight but have

not tried to do so within the last year and half have tried to lose weight without consulting a healthcare professional.[10]

1.1 Current treatment landscape

Current guidelines for obesity management recommend determining the degree to which an individual is overweight or has obesity and, depending on the severity, applying multicomponent interventions.[11-16] Lifestyle modifications are recommended for all patients who require weight loss, whereas additional pharmacotherapy is advised for individuals for whom lifestyle interventions have failed.[11-16] Lifestyle modifications can include reduced energy intake (typically to achieve an energy deficit of ≥ 500 kcal/day), increased aerobic physical activity levels to ≥ 150 minutes/week and behavioural change strategies to facilitate adherence to diet and physical activity (self-monitoring and reporting of dietary intake, physical activity and weight measurements).[11-15] A variety of diets designed to reduce energy intake may successfully result in weight loss in adults who are overweight or affected by obesity. Meal plans including Mediterranean-style or vegetarian/vegan-style diets, which are higher in plant-based foods including olive oil (rich in monounsaturated oleic acid) and lower in processed food and meat than typical Western

diets, may promote weight loss and cardiovascular benefits that are similar to those associated with low-fat diets (25–30% of calorie intake from fat).[11, 14] Notably, in the Dietary Intervention-Randomized Controlled Trial (DIRECT), a low-fat diet in people with type 2 diabetes (T2DM) elicited a lower mean weight loss (2.9 kg) compared with a Mediterranean (4.4 kg) or a low-carbohydrate (4.7 kg) diet~~, while improving lipid profile and glycaemic control to a greater extent~~.[17] Compared with the low-fat diet, the low-carbohydrate diet improved lipid profiles, while the Mediterranean diet decreased fasting plasma glucose levels in patients with diabetes.[17] A recent randomised, controlled trial also showed that a 6-week low-carbohydrate diet, with high intake of protein and fat and energy intake adjustments to ensure weight stability, improved glycaemic control and reduced liver fat content in patients with diabetes.[18] These observations suggest that it is not necessarily fat intake that is responsible for increased fat deposition. Intermittent fasting has also gained interest for the treatment of obesity and diabetes, and has been recommended to comprise regular periods of no or very limited calorie intake (< 25% of calorie requirement), for example, a 16-hour daily fast or a 24-hour fast on alternate days or two non-consecutive days in a week.[19] On non-fasting days, calorie intake can be unrestricted. A systematic review of 27 trials of people who were overweight or affected by obesity demonstrated that intermittent fasting reduces bodyweight by 0.8–13% in the short

term (2–52 weeks), regardless of change in calorie intake.[19] In studies of patients with concurrent obesity and T2DM, improved glycaemic control was also reported with intermittent fasting.[19]

With dietary interventions, most patients will reach a plateau in bodyweight loss at approximately 6–12 months, ranging from 3–12 kg, then will slowly regain weight over 2–5 years, with total weight loss reducing to 0 to 3–4 kg.[11, 12] This pattern is most likely due to the progressive reduction of energy expenditure associated with bodyweight loss and the reduction of lean body mass. Therefore, long-term bodyweight loss requires adjustment of lifestyle modifications over time. Adults who are unable to achieve or sustain bodyweight loss with comprehensive lifestyle modifications, who have either a BMI ≥ 30 kg/m^2 or ≥ 27 kg/m^2 with one or more comorbidities, can be considered for adjunct pharmacologic therapy.[11-13]

US Food and Drug Administration-approved agents for the treatment of obesity include appetite suppressants, such as glucagon-like peptide-1 receptor (GLP-1R) agonists (liraglutide and semaglutide), ~~serotonin receptor agonists and~~ noradrenergic drugs (phentermine/topiramate and naltrexone/bupropion), and pancreatic lipase inhibitors (orlistat).[20, 21] Phentermine stimulates noradrenaline release which in turn suppresses appetite, augmented by topiramate, an anticonvulsant.[22] Across randomised controlled

trials, a mean bodyweight loss of 9.8 kg was observed with phentermine/topiramate treatment.[23] Naltrexone acts as an opioid antagonist and bupropion as a dopamine and noradrenaline reuptake inhibitor, the combination of which promotes satiety and increased energy expenditure leading to a mean bodyweight loss of 4.4 kg.[23, 24] Orlistat is a selective pancreatic lipase inhibitor that moderates intestinal absorption and digestion of fat, with an observed mean bodyweight loss of 3.1 kg.[22, 23] A 2-year study showed an additional bodyweight loss of ≥ 5% with the GLP-1R agonist liraglutide, which was significantly greater, by 3.0 kg ($p<0.001$), than weight loss with ~~the pancreatic lipase inhibitor~~ orlistat.[25] In this trial, bodyweight loss stabilised by approximately 36 weeks,[25] similar to that seen in trials with orlistat or the noradrenergic drug sibutramine.[26, 27] Previous pharmacological agents approved for the treatment of obesity, including amphetamine derivatives, cannabinoid receptor blockers and serotonin reuptake inhibitors, have been withdrawn due to their adverse event (AE) profiles (TABLE 1).[22, 28]

Bariatric surgery is an option for individuals with either a BMI ≥ 40 kg/m^2 or ≥ 35 kg/m^2 and with comorbidities for whom appropriate non-surgical methods have failed.[11-16, 31] Roux-en-Y gastric bypass, often called gastric bypass, has traditionally been considered the gold standard bariatric procedure for weight loss. The underlying mechanisms are loss of appetite resulting in reduced food intake, most likely driven by the

exaggerated secretion of gut hormones that occurs a few days after surgery. The increased secretion of these hormones, including glucagon-like peptide-1 (GLP-1) and peptide YY (PYY), is due to accelerated exposure and absorption of nutrients in the small intestine.[32-34] Changes in anatomy leading to mechanical restriction of food intake and malabsorption of macronutrients were originally thought to be responsible for weight loss following bariatric surgery. However, these effects have since been found to be inappreciable,[34] except with less commonly used procedures such as jejunoileal bypass, biliopancreatic diversion and duodenal switch, which dramatically reduce intestinal resorption of nutrients. The mode of action of gastric sleeve operations, now the most widely used procedure to treat obesity,[35] is not fully elucidated, but the accelerated passage of nutrients into the small intestine, which also leads to exaggerated gut hormone secretion, is thought to play a role.[36] Most surgical procedures are, in principle, irreversible and are not without complications;[37] moreover, surgical intervention alone is unlikely to manage obesity in the majority of patients. Therefore, there is a large unmet medical need for a highly efficacious pharmacological agent with a favourable risk–benefit profile for the treatment of obesity, especially in chronically ill patients with concomitant disease (e.g. hypertension, T2DM and chronic obstructive pulmonary disease).

1.2. Rationale for targeting the incretin/glucagon system in obesity

Energy balance is maintained by an intricate network of interacting feedback mechanisms involving the hypothalamus, the brainstem, higher brain centres and, in the periphery, the stomach, gut, liver, thyroid, endocrine pancreas and adipose (fat) tissue.[38] Hormones from peripheral tissues such as leptin, ghrelin, cholecystokinin, pancreatic polypeptide, PYY (PYY3-36), GLP-1 and oxyntomodulin have been shown to regulate appetite.[39-47] Resistance to the actions of some of these hormones appears to be associated with common obesity. For example, leptin is secreted by adipose tissue and is thought to be a key peptide in reducing food intake based on the extreme obesity that develops in the absence of leptin signalling.[38, 48] However, people affected by obesity have chronically elevated leptin levels and are resistant to its anorexigenic effects[39, 48]—this is thought to be caused, in part, by downregulation of a feedback loop by the high leptin levels.[49] Food intake is also regulated by the mesolimbic reward system and has been shown to activate some of the same circuits involved in drug addiction.[38, 50-52]

The pathophysiology of obesity is complex and currently approved therapies for obesity only target a few of the many pathways involved; thus, single-targeting agents have limited efficacy.[22, 53] An integrated approach to the treatment of obesity that targets multiple mechanisms such as feeding circuits, glucose metabolism and energy expenditure,

is therefore assumed to be more effective than single-targeting agents.[53] Proglucagon-derived peptides, glucagon and the incretin hormones GLP-1 and glucose-dependent insulinotropic polypeptide (GIP), represent attractive targets for managing obesity and metabolic disorders[53-56] because they may play a direct role in multiple mechanisms involved in the disease, including satiety, energy homeostasis and lipolytic activity.[46, 57-59]

Dipeptidyl peptidase-4 (DPP-4) inhibitors, approved for use in T2DM,[60] prevent DPP-4 from cleaving various gut peptides including GLP-1 and GIP;[22, 61] however, levels of GLP-1 activity achieved by DPP-4 inhibitors alone are not sufficient to stimulate a decrease in bodyweight.[22, 61, 62] Furthermore, DPP-4 inhibition stops the conversion of PYY 1-36 to PYY 3-36, the molecular form that reduces appetite and food intake,[63] and this may further limit the effects on bodyweight loss since what is gained with respect to the effects of GLP-1 (and GIP) is lost with respect to the effects of PYY.[32]

GLP-1 has a short half-life and is cleaved by DPP-4 and neutral endopeptidase within 1.5–2 minutes. This has led to the development of GLP-1R agonists that have higher enzymatic stability towards both peptidases than endogenous GLP-1, resulting in slower elimination.[62] However, since the peptide is also cleared by the kidneys, prolongation techniques have been developed to ensure lasting agonism. For example, the GLP-1R

agonist liraglutide is acylated and its acyl moiety (palmitic acid) binds to albumin, whereby the peptide survives in the circulation.[64] This agonist has been shown to effectively cause bodyweight loss in humans and experimental animals, in which sufficient levels of the natural peptide do not remain in the circulation to account for this effect.[65-67] Investigations using rat models demonstrate that liraglutide may cross the blood–brain barrier via the circumventricular organs (the area postrema, the subfornical organ, the choroid plexus and the median eminence) and reach, for instance, the arcuate nucleus.[67] Here, liraglutide could activate neurons expressing proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART), which are key appetite-regulating neurons, and indirectly inhibit neurotransmission in neurons expressing neuropeptide Y (NPY) and agouti-related peptide (AgRP) via GABA-dependent signalling.[67] Other long-acting GLP-1R agonists that target the gastrointestinal (GI) tract and central nervous system, (CNS) including dulaglutide, exenatide extended-release and semaglutide, have since been developed that reduce bodyweight to a similar (~2–3%) or, in the case of injectable semaglutide, greater (~4–6%) extent as liraglutide with a similar tolerability profile in humans.[68]

As GLP-1, GIP and glucagon have related peptide sequences, it is possible to create analogues with agonist activity at more than one receptor type, for instance, combining GLP-

1R agonist activity with the effects of glucagon and/or GIP.[61] Here, we discuss pre-clinical and clinical findings in obesity and other therapeutic areas of interest for glucagon, the endogenous incretin hormones GIP and GLP-1 and GLP-1R agonists, as well as their actions when combined as dual and triple agonists.

2. GLUCAGON IN OBESITY

Glucagon is a pancreatic hormone, with receptors predominantly expressed in the liver. There also appear to be receptors expressed in the kidneys (although the localisation is uncertain), while expression in the heart, adipose tissue, CNS, adrenal gland and spleen is variable and may be species dependent (

FIGURE 1).[66]

Glucagon regulates amino acid metabolism and is released from alpha cells following amino acid stimulation as part of the liver–alpha cell axis.[69-71] In addition, glucagon has long been recognised to regulate glucose homeostasis, counteracting the actions of insulin by stimulating hepatic glucose production (glycogenolysis and gluconeogenesis).[61] Glucagon, at least at pharmacological doses, may regulate lipid metabolism, energy expenditure and food intake in multiple species.[54, 58, 72-76] In humans, hepatic fat

synthesis is suppressed after glucagon administration.[54] Glucagon stimulates beta-oxidation of fatty acids and inhibits the formation of malonyl-coenzyme A, the first intermediate of fatty acid synthesis.[77] However, the extent to which glucagon influences whole-body lipid metabolism, particularly in individuals affected by obesity, remains controversial.[58, 77] In rodents, glucagon has been shown to stimulate lipolysis in adipocytes,[78-80] however glucagon receptor expression has not been successfully demonstrated in human adipocytes.[77] The potential lipolytic effect of glucagon in humans has only been shown *in vitro* and at concentrations much higher than physiological levels in plasma.[77] Glucagon may also increase energy expenditure by inducing thermogenesis in brown adipose tissue (BAT), as shown in humans and in animal models.[81-83] This thermogenic effect is thought to be mediated through activity of the sympathetic nervous system, given that inhibiting β-adrenergic activity impairs the ability of glucagon to increase energy expenditure.[84] However, the contribution of thermogenesis to overall energy expenditure remains unknown, and this effect may be too small to result in bodyweight loss.[75] In animal models, glucagon reduces food intake when administered peripherally and into the CNS.[56, 66, 85, 86]. Because of the extremely short half-life of glucagon in rodents,[87] long-acting glucagon analogues are likely to be more effective. Glucagon infused into the hepatic portal vein reduces spontaneous meal size in rats.[85] Conversely,

infusion of anti-glucagon antibodies into the hepatic portal vein increases spontaneous meal size in rats.[85, 88] These observations have led to the suggestion that glucagon may act in the liver to generate a satiety signal that is relayed to the brain via the hepatic branch of the vagus nerve.[85] Glucagon infusion at pharmacological doses in humans has been demonstrated to increase, rather than decrease, respiratory quotient and carbohydrate oxidation.[81] However, increases in energy expenditure have been reported at doses that did not activate the sympathetic nervous system.[89]

In patients with diabetes, levels of glucagon are elevated during fasting and, in response to carbohydrate ingestion, the normal suppression is delayed or even briefly reversed. These abnormalities are important for the development of diabetic hyperglycaemia, as indicated by the results of glucagon receptor (GCGR) antagonist administration, which may normalise glucose levels.[90] However, as a therapy for T2DM, GCGR antagonists have shown undesirable AEs including elevated liver enzymes, accumulation of liver triglycerides and hyperglucagonaemia, which have discouraged further development of GCGR antagonists in this patient population.[61] Inappropriate glucagon secretion and regulation has been shown in patients with obesity, as well as those with NASH.[71, 91-93]. The inappropriate elevation of circulating glucagon is likely the consequence of increased levels of plasma amino acids, representing a disruption of the

liver–alpha cell axis caused by hepatic fat accumulation.[71, 94] Hepatic steatosis can lead to glucagon resistance, wherein glucagon-induced amino acid metabolism is impaired causing elevated plasma levels amino acids and hence also glucagon.[71] Indeed, it may be that among patients with T2DM, those with non-alcoholic fatty liver disease (the vast majority) and hyperaminoacidemia also have hyperglucagonemia.[92] This disruption of the liver–alpha cell axis is mainly due to the accumulation of intrahepatic lipid and may contribute to the development of T2DM, rather than being a consequence of it.[71, 92]

3. GLP-1 IN OBESITY

GLP-1, an incretin hormone secreted from the L cells in the small intestine after food intake, stimulates insulin secretion (in a glucose-dependent manner) and regulates energy intake.[46, 95-97] GLP-1 is also produced in the caudal portion of the nucleus of the solitary tract, a region receiving afferent input from the GI tract.[98, 99] GLP-1 acts on peripheral and central receptors in the gut and brain to delay gastric emptying, inhibit GI secretion and decrease food intake through activation of satiety pathways and efferent pathways regulating GI function (Figure 2).[66, 67, 95, 100, 101] GLP-1 also reduces glucagon secretion by alpha cells, thereby inhibiting hepatic glucose production.[102, 103] The GLP-1R agonist liraglutide

has been shown to reduce bodyweight in patients with prediabetes and in those with obesity,[104] and has been approved for weight management in adults with obesity as an adjunct to a reduced-calorie diet and increased physical activity.[65] In addition, results from the STEP 3 trial demonstrate that the GLP-1R agonist semaglutide reduces bodyweight in adults with obesity.[105]

4. GIP IN OBESITY

GIP, an incretin hormone secreted from K cells in the upper gut, acts in concert with GLP-1 to exert 'the incretin effect', resulting in substantial physiological stimulation of insulin secretion after glucose administration.[62, 106-108] In contrast with GLP-1, GIP may stimulate glucagon secretion at lower glucose levels.[62] Although the insulinotropic activity of GIP has now been confirmed in human studies involving a GIP receptor (GIPR) antagonist,[59, 109] whether GIP contributes to the development of obesity remains a controversial concept.[110] Mice lacking the GIPR are protected from diet-induced obesity and crossing of GIPR-null mice with obese ob/ob mice reduces adiposity.[111, 112] However, other studies have demonstrated a reduction in calorie intake and bodyweight after both central and peripheral administration of GIPR agonists.[113, 114] This effect is

potentially mediated by GIP-recruited neuropeptides linked to regulation of food intake and energy balance.[115] GIP does not appear to have any acute effects on food intake in humans,[116] yet discussions are ongoing on the role of GIPR agonists and antagonists as weight loss agents.[117]

5. DUAL GLP-1R/GCGR AGONISTS

In animal models of obesity, administration of dual GLP-1R/GCGR agonists resulted in superior weight loss, lower glucose levels and reduced food intake compared with pure GLP-1R agonists alone.[118-121] Weight loss with a dual GLP-1R/GCGR agonist was maintained over 7 days, whereas the effect of a pure GLP-1R agonist alone plateaued mid-week before returning to vehicle control level by Day 7.[119] In humans, dual GLP-1R/GCGR agonism is thought to result in additive effects of reducing food intake and lowering glucose levels, making this an attractive approach for weight management in individuals with diabetes. In a Phase II trial, individuals with diabetes and who were overweight or affected by obesity receiving the dual GLP-1R/GCGR agonist cotadutide (MEDI0382) achieved significant lowering of glucose levels and bodyweight loss compared with patients receiving placebo over 41 days ($p<0.0001$ and $p=0.0008$, respectively).[122] Decreased appetite occurred more frequently in patients receiving cotadutide than those receiving placebo (20% vs. 0%),

however, GI disorders were also more frequent (74% vs. 40%).[122] Overall, the proportion of patients experiencing treatment-emergent AEs was similar in both groups (88% vs. 88%).[122] In a Phase IIb trial of cotadutide in patients with overweight/obesity and T2DM, significant reductions in glycated haemoglobin levels ($p<0.001$) and percentage of bodyweight ($p<0.001$) were observed at all tested doses (100, 200 or 300 μg) of cotadutide versus placebo, and significant reductions in the percentage of bodyweight were seen with 300 μg cotadutide versus liraglutide ($p=0.009$).[123] In addition, treatment with cotadutide improved hepatic parameters, with decreases in alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase and procollagen III levels and improvements in non-alcoholic fatty liver disease fibrosis score and Fibrosis-4 index observed in comparison with placebo, whereas liraglutide had no notable effect.[123] The incidence of treatment-emergent AEs was higher across all doses of cotadutide compared with placebo and liraglutide, with GI disorders being most commonly reported.[123] In overweight individuals without diabetes, dual GLP-1/glucagon infusion increased energy expenditure to a similar degree as glucagon alone; however, the addition of GLP-1 reduced the hyperglycaemic effect of glucagon.[124] Dual GLP-1/glucagon infusion has been reported to significantly reduce food intake (−13%, $p<0.05$) compared with similar doses of GLP-1 and glucagon administered separately, although patients reported post-prandial nausea and

some vomiting.[124] A trend towards increased pulse rate was also seen with dual GLP-1/glucagon infusion compared with placebo or GLP-1 alone, although no substantial change in blood pressure was recorded.[81] Thus, concomitant GCGR and GLP-1R activation provides the beneficial effects of glucagon (i.e., maintaining a significant reduction in food intake with little effect on plasma glucose levels; Figure 2).[81, 124]

6. DUAL GLP-1R/GIPR AGONISTS

Although the lipogenic potential of GIP alone is under debate, coactivation of GLP-1R and GIPR is an attractive prospect in the treatment of T2DM and perhaps obesity (Figure 2).[125] For example, GIP analogues that do not alter bodyweight when administered alone to mice with diet-induced obesity were found to enhance GLP-1-induced weight loss, reduce food intake and prevent fat mass accumulation;[126, 127] however, similar results have also been obtained with GIP antibodies.[128] The dual GLP-1R/GIPR agonist tirzepatide (LY3298176) has been shown to improve insulin sensitivity independently of GLP-1R induced weight loss in *Glp-1r*-null mice (i.e. via GIPR antagonism), but whether this effect is present in man remains to be seen.[129] Furthermore, a balanced unimolecular GLP-1R and GIPR agonist reduced bodyweight, food intake and fat mass in mice with diet-induced obesity to a greater

extent than liraglutide.[127] Although the exact mechanisms of GLP-1/GIP synergism are unclear, it has been hypothesised that GIP could act directly via the CNS by inhibiting food intake, enhancing the anorexigenic action of GLP-1 or by increasing tolerability to GLP-1R agonists.[130] Dual GLP-1R/GIPR agonism has also shown efficacy in humans. In a Phase II trial of ~~the dual GLP-1R/GIPR agonist~~ tirzepatide ~~(LY3298176)~~, more individuals with T2DM achieved weight loss of ≤ 5% and ≤ 10%, and glucose control with the dual GLP-1R/GIPR agonist than with a GLP-1R agonist (dulaglutide) alone.[131] Decreased appetite (although desirable) was the second most common AE, with dose-related GI events being the most common but the majority being transient and mild to moderate in severity.[131] In the Phase III SURPASS-2 trial, treatment with tirzepatide was superior to semaglutide at reducing bodyweight in patients with T2DM at all tested doses (5, 10 or 15 mg), with 34–57% of patients receiving tirzepatide experiencing bodyweight reductions of ≥10%, compared with 24% of those receiving semaglutide (1 mg).[132] In~~itial, unpublished data from~~ the Phase III SURPASS-3 ~~and SURPASS-5~~ trial~~s~~ of tirzepatide (5, 10 or 15 mg) in individuals with T2DM (with or without metformin and/or a SGLT-2 inhibitor) ~~show~~ bodyweight reduction ranged from –9.8 kg to –15.2 kg ~~loss of up to 13.9% and 11.6%, respectively, for tirzepatide compared with 2.7% and 1.7%, respectively, for placebo~~.[133] ~~In both trials, t~~ The most commonly reported AEs in the tirzepatide arms were GI-related and generally mild to

moderate in severity, with up to ~11% of participants in the tirzepatide arms discontinuing treatment due to AEs.[134]

7. TRIPLE GLP-1R/GCGR/GIPR AGONISTS

The synergistic actions of glucagon to reduce food intake and increase energy expenditure, GLP-1 to reduce calorie intake and GIP to potentiate bodyweight loss may aid in the treatment of obesity (Figure 2). The addition of both incretin components to glucagon appear to better mitigate the hyperglycaemic action of glucagon compared with the presence of GLP-1 or GIP alone, allowing for greater glucagon dosing and therefore greater potential for weight loss.[135] In animal models of obesity, balanced unimolecular triple agonism proved superior to existing dual agonists and best-in-class mono-agonists in reducing bodyweight and enhancing glycaemic control.[136] In a murine model of diet-induced NASH and fibrosis, the triple combination of GLP-1R, GCGR and GIPR mono-agonists increased bodyweight loss, reduced liver triglycerides and improved histological NASH disease activity score; weight loss was similar to that obtained with liraglutide alone, but histological NASH disease activity score was significantly improved ($p<0.01$)[137]. In addition, HM15211, a long-acting

triple agonist peptide, reduced bodyweight and improved liver function in cynomolgus monkey models of obesity and NASH.[138]

8. BALANCED AGONISM, SPECIFICITY AND SELECTIVITY

Activation of multiple receptors can be achieved by either a combination of two or more different monoagonists or a unimolecular multiagonist. A multiagonist may take the form of a multivalent fusion of monoagonist analogues or a hybridised molecule comprising multiple epitope regions that has an overall size comparable to the native peptides.[61] The latter approach is favoured when targeting GLP-1R, GCGR and/or GIPR because they are the same type of receptor (class B G-protein coupled) and have a high degree of sequence homology and native ligands with similar secondary structures.[61] The GCGR, GIPR and especially GLP-1R exhibit cross-reactivity with the other's ligands, with glucagon being the most cross-reactive ligand;[61] thus, a full investigation and characterisation of the interactions at the relevant receptors is required. For example, LY2409021, originally developed as a GCGR antagonist, was subsequently found to block the actions of glucagon at the GCGR and GLP-1R, the actions of GLP-1 at the GLP-1R and the actions of GIP at the GIPR *in vitro*.[139] When designing unimolecular dual and triple agonist peptides, it is

important to consider whether the molecule activates all target receptors with equal potency (balanced agonism) or has a higher affinity for one receptor over the other(s) (preferential agonism).[61] An appropriately balanced unimolecular agonist can only occupy a single receptor at a time, which theoretically reduces the likelihood of preferential binding at any one type of receptor, as could happen with a multivalent fusion of agonists with different affinities.[61] In addition, the selectivity of an agonist for a given receptor has relevance for predicting and, ultimately, avoiding off-target effects.[139]

9. AGENTS TARGETING THE INCRETIN/GLUCAGON SYSTEM IN OBESITY

The synergy of dual and triple incretin agonists in increasing bodyweight loss through decreased appetite and increased energy expenditure may offer an advanced therapeutic option for patients with obesity, and several novel unimolecular peptides are in clinical development (TABLE 2). Most trials have yet to be fully published and the majority of published reports describe early pharmacokinetic and tolerability studies; nevertheless, trials of GG-co-agonist 1177, JNJ-6456511, BI 456906 and tirzepatide are currently investigating bodyweight-related outcomes.

10. SAFETY

Glucagon and related peptides have a multitude of hormonal and metabolic effects that are not all desirable when targeting the receptors therapeutically.[66] Some unwanted effects are usually classified as GI, although it is likely that they are mainly due to interactions with central receptors. Whereas delayed gastric emptying may be sensed as fullness, one consequence of the interaction with area postrema receptors triggered by GLP-1 and glucagon appears to be mild-to-moderate transient nausea,[46, 124, 131] which has also been reported in studies of single GLP-1 agonists in patients with diabetes.[25, 104] Additional GI AEs (vomiting and diarrhoea) have been observed in trials of GLP-1R/GCGR dual agonists.[122, 131] Cardiovascular AEs are of potential concern, and a number of cardiovascular outcomes trials will be required as development proceeds, such as the ongoing SURPASS-CVOT of tirzepatide.[140] Completed trials of the GLP-1R agonists liraglutide, semaglutide and dulaglutide have demonstrated superiority with respect to rates of adverse cardiac outcomes in comparison with placebo.[141-143]

11. CONCLUSIONS

Obesity is associated with a considerable and progressive disease burden and an effective pharmacologic intervention is lacking. Glucagon is an attractive target for bodyweight management in individuals with obesity due to its ability to reduce food intake and stimulate energy expenditure, potentially without cardiovascular AEs. However, its action may need to be counterbalanced by concomitant use of incretin hormones (i.e., preventing hyperglycaemia and enhancing the central effects of glucagon). The incretin hormone GLP-1 is also an attractive target because it supresses appetite and reduces food intake, although the role of the incretin hormone GIP in bodyweight reduction is under debate. GIPR agonism alone has been shown to reduce bodyweight in mice with obesity, as observed with GIPR agonists with a longer half-life than endogenous GIP. However, these agents alone may have limited efficacy. It is reasonable to assume that the dual and triple combinations of glucagon and incretin hormone receptor agonists could provide superiority in maximising bodyweight loss. Unimolecular dual and triple agonists that target glucagon and incretin hormone receptors have been shown to improve bodyweight loss, lower glucose levels and reduce food intake in animal models of obesity and NASH, and multiple dual agonists are in clinical development for the treatment of obesity and diabetes. Phase II clinical data have established that the dual GLP-1R/GIPR agonist tirzepatide has superior antidiabetic efficacy

compared with the GLP-1R agonist dulaglutide, alongside reductions in bodyweight and the induction of satiety. Reductions in bodyweight and glucose levels have also been demonstrated with dual GLP-1R/GCGR agonists. The extent to which glucagon contributes to such treatment effects remains to be understood, but it may contribute to weight loss by reducing appetite and food intake, while concomitant GLP-1R agonism ensures normal glucose control. Further research is required to fully understand the molecular mechanisms of action that underpin the efficacy of both dual and triple receptor agonists and the respective metabolic effects.

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Table and figure legends

TABLE 1 Previous pharmacological agents approved for the treatment of obesity and the AEs resulting in their withdrawal[28-30]

*Approved for use up to 12 weeks

Abbreviations: AE, adverse event; NDRA, noradrenaline–dopamine releasing agent; NDRI, noradrenaline–dopamine re-uptake inhibitor; NRA, noradrenaline releasing agent; SNDRA, serotonin–noradrenaline–dopamine releasing agent; SNRI, serotonin–noradrenaline re-uptake inhibitor; SRI, serotonin re-uptake inhibitor.

TABLE 2 Summary of clinical trials of agents targeting the incretin/glucagon system under investigation in patients with obesity

Abbreviations: BMI, body mass index; BP, blood pressure; EE, energy expenditure; FFF, free fatty acids; HbA1c, glycated haemoglobin; MACE, major adverse cardiac events; PK, pharmacokinetics; QOL, quality of life; RQ, respiratory quotient; T2DM, type 2 diabetes; VAS, visual analogue score; WC, waist circumference.

FIGURE 1 Physiological and pharmacological actions of glucagon. Glucagon has a number of physiological (blue), pharmacological (green) and hypothetical (orange) actions in several organs, some of which may be species dependent. GI, gastrointestinal.

FIGURE 2 Incretin/glucagon targeting agents achieve their weight loss effect through a variety of mechanisms in several organs. GCG, glucagon; GI, gastrointestinal; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1.

TABLE 1

Agent	Mechanism of action	Launch date	Withdrawal date	Reason for withdrawal
Amfepramone (diethylpropion)	SNDRA	1957	1975	Cardiotoxicity
Amphetamine	SNDRA	1939	1973	Drug abuse/ dependence
Aminorex fumarate	SRI	1962	1967	Cardiotoxicity

Benfluorex	SRI	1976	2009	Cardiotoxicity
Caffeine and Ephedra	Non-selective adrenergic agonist	1994	2004	Cardiotoxicity, psychiatric
Chlorphentermine	SRI	1962	1969	Cardiotoxicity
Clobenzorex	SNDRA	1966	2000	Drug abuse, psychiatric
Cloforex	SRI	1965	1967	Cardiotoxicity
Cyclovalone + retinol + tiratricol	Bile acid secretion	1964	1988	Hepatotoxicity
Dexfenfluramine	SRI	1995	1997	Cardiotoxicity
Fenbutrazate	NDRA	1957	1969	Drug abuse, psychiatric
Fenfluramine	SRI	1973	1997	Cardiotoxicity
Fenproporex (perphoxene)	NRA	1966	1999	Drug abuse, psychiatric
Iodinated casein strophanthin	Thyroxine analogue	1944	1964	Endocrine, metabolism
Levoamphetamine	SNDRA	1944	1973	Drug abuse/ dependence
Lorcaserin	Serotoninergic agonist	2012	2020	Increased risk of cancer
Mazindol	NDRA	1970	1987	Drug abuse, psychiatric (interaction with lithium)
Mefenorex (methylphenethylamine)	SNDRA	1966	1999	Drug abuse, psychiatric
Methamphetamine (desoxyephedrine)	SNDRA	1944	1973	Drug abuse/ dependence
Phendimetrazine	NDRA	1961	1982	Drug abuse
Phenmetrazine	NDRA	1956	1982	Drug abuse
Phentermine*	NDRA	1959	1981	Drug abuse
Phenylpropanolamine (norpseudoephedrine)	NDRA	1947	1987	Haemorrhagic stroke
Pipradrol	NDRI	1953	1982	Drug abuse

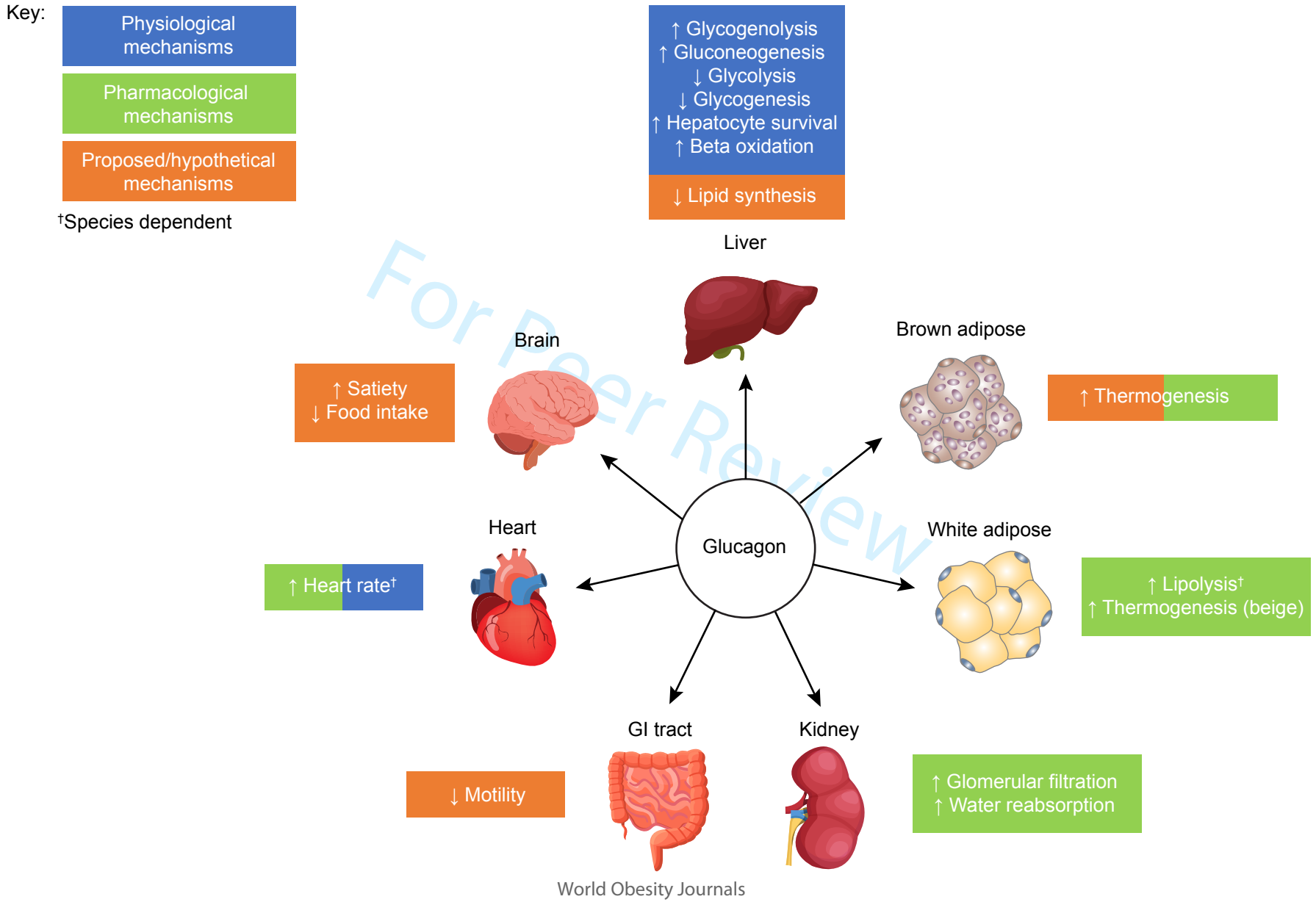
Pyrovalerone	NDRA	1974	1979	Drug abuse
Rimonabant	Cannabinoid antagonist/inverse agonist	2006	2007	Psychiatric
Sibutramine	SNRI	2001	2002	Cardiotoxicity, psychiatric

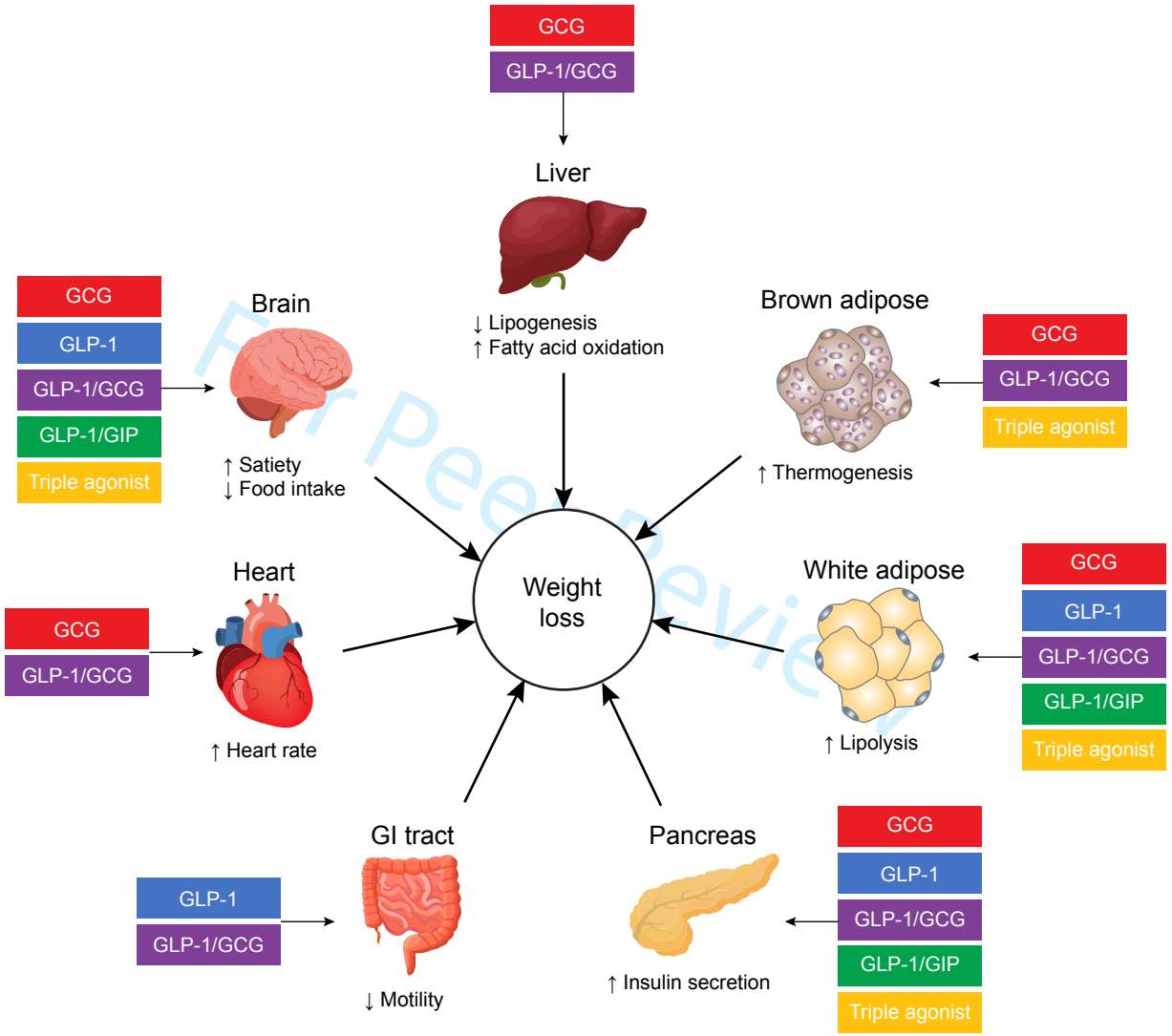
TABLE 2

Agonist	Agent	Trial phase	Selected outcome measures	Trial number
Single agonists				
GCGR agonist	NN9030	Phase I	PK/safety	NCT02235961
		Phase I	PK/safety; Δ HbA1c	NCT02870231
		Phase I	PK/safety; Δ HbA1c	NCT02835235
Dual agonists				
GLP-1R/GCGR agonists	GG-co-agonist 1177	Phase I	PK/safety; Δ bodyweight	NCT02941042
		Phase I	PK/safety	NCT03308721
	JNJ-6456511	Phase I	PK/safety	NCT03586843
		Phase II (T2DM)	Δ bodyweight; ≥ 5% bodyweight loss	NCT03586830
		Phase II	Δ bodyweight; ≥ 5% and ≥ 10% bodyweight loss	NCT03486392
	MOD 6031	Phase I	PK/safety	NCT02692781
	BI 456906	Phase I	PK/safety	NCT03591718
		Phase I	PK/safety	NCT04384081

		Phase II	Δ bodyweight; ≥ 5%, ≥ 10% and ≥ 15% bodyweight loss	NCT04667377
		Phase II (T2DM)	Δ HbA1c; Δ bodyweight; ≥ 5%, ≥ 10% bodyweight loss	NCT04153929
GLP-1R/GIPR agonists	Tirzepatide (LY3298176)	Phase I	Δ food intake; Δ EE; Δ RQ; Δ % body fat; Δ FFA; Δ post-meal glucose	NCT04081337
		Phase I	Δ energy intake; Δ appetite VAS	NCT04311411
		Phase I (+/– T2DM)	PK; Δ HbA1c	NCT04407234
		Phase III (T2DM)	Δ bodyweight; ≥ 5%, ≥ 10% and ≥ 15% bodyweight loss; Δ WC; Δ BMI; Δ fasting glucose and insulin; Δ HbA1c; Δ lipids; Δ BP; Δ QOL	NCT04657003
		Phase III	Δ bodyweight; ≥ 5%, ≥ 10% and ≥ 15% bodyweight loss; Δ WC; Δ BMI; Δ fasting glucose and insulin; Δ HbA1c; Δ lipids; Δ BP; Δ QOL	NCT04657016
		Phase III	Δ bodyweight; ≥ 5% and ≥ 10% bodyweight loss; Δ WC; Δ BMI; Δ fasting glucose and insulin; Δ HbA1c, Δ lipids; Δ BP; Δ QOL	NCT04660643
		Phase III	MACE	NCT04255433
		Phase III	Δ bodyweight; ≥ 5%, ≥ 10% and ≥ 15% bodyweight loss; Δ WC; Δ BMI;	NCT04184622

			Δ fasting glucose and insulin; time to T2DM onset; Δ HbA1c; Δ lipids; Δ BP; Δ QOL	
Triple agonists				
GLP-1R/ GCGR/GIPR agonists	Tri-agonist 1706	Phase I	PK/safety	NCT03095807
		Phase I	PK/safety	NCT03661879
	HM15211	Phase I	Safety	NCT03374241
		Phase I	Safety	NCT03744182





Dear Professor York,

Further to our correspondence last year, we are pleased to submit our review article entitled **The incretin/glucagon system as a target for pharmacotherapy of obesity** for consideration for publication in *Obesity Reviews*.

As we outlined in our enquiry, our review discusses the complexities of obesity, focusing on the involvement of the incretin/glucagon system and the rationale for targeting it to achieve weight loss. We review and discuss relevant pre-clinical and clinical findings in obesity and other therapeutic areas of interest for glucagon, endogenous incretins GIP and GLP-1, including GIP receptor antagonists and GLP-1 receptor agonists, as well as their combinations as dual and triple agonists. We then summarise current pharmacotherapies in development that target multiple mechanisms in the incretin/glucagon system and their potential for achieving weight loss in obesity.

Thank you for your kind consideration.

Yours sincerely,

Stefano Del Prato, Baptist Gallwitz, Jens Juul Holst, Juris Meier