

Peptide-based multi-agonists: a new paradigm in metabolic pharmacology

■ S. J. Brandt^{1,2}, T. D. Müller^{1,2}, R. D. DiMarchi³, M. H. Tschöp^{1,2,4} & K. Stemmer^{1,2}

From the¹Institute for Diabetes and Obesity, Helmholtz Diabetes Center, Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH); ²German Center for Diabetes Research (DZD), Neuherberg, Germany; ³Department of Chemistry, Indiana University, Bloomington, IN, USA; and ⁴Division of Metabolic Diseases, Technische Universität München, Munich, Germany

[Content List](#) - Read more articles from the symposium: Neuroendocrine Interfaces in Physiology and Disease

Abstract. Brandt SJ, Müller TD, DiMarchi RD, Tschöp MH, Stemmer K (Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), German Center for Diabetes Research (DZD), Neuherberg, Germany; Indiana University, Bloomington, IN, USA; Technische Universität München, Munich, Germany). Peptide-based multi-agonists: a new paradigm in metabolic pharmacology (Review Symposium). *J Intern Med* 2018; **284**: 581–602.

Obesity and its comorbidities, such as type 2 diabetes, are pressing worldwide health concerns. Available anti-obesity treatments include weight loss pharmacotherapies and bariatric surgery. Whilst surgical interventions typically result in significant and sustained weight loss, available pharmacotherapies are far less effective, typically decreasing body weight by no more than 5–10%. An emerging class of multi-agonist drugs may eventually bridge this gap. This new class of specially tailored drugs hybridizes the amino acid sequences of key metabolic hormones into one single entity with enhanced potency and sustained action. Successful examples of this strategy include multi-agonist drugs targeting the receptors

for glucagon-like peptide-1 (GLP-1), glucagon and the glucose-dependent insulinotropic polypeptide (GIP). Due to the simultaneous activity at several metabolically relevant receptors, these multi-agonists offer improved body weight loss and glucose tolerance relative to their constituent monotherapies. Further advancing this concept, chimeras were generated that covalently link nuclear acting hormones such as oestrogen, thyroid hormone (T₃) or dexamethasone to peptide hormones such as GLP-1 or glucagon. The benefit of this strategy is to restrict the nuclear hormone action exclusively to cells expressing the peptide hormone receptor, thereby maximizing combinatorial metabolic efficacy of both drug constituents in the target cells whilst preventing the nuclear hormone cargo from entering and acting on cells devoid of the peptide hormone receptor, in which the nuclear hormone might have unwanted effects. Many of these multi-agonists are in preclinical and clinical development and may represent new and effective tools in the fight against obesity and its comorbidities.

Keywords: diabetes, glucagon, GIP, GLP-1, multi-agonism, peptides.

Introduction

Obesity is a growing public health problem that imposes a large economic burden on our society. In 2015, 107.7 million children and 603.7 million adults worldwide were classified as obese [1]. Obesity is one of the most important and modifiable risk factors for the development of metabolic complications such as type 2 diabetes (T2D), cardiovascular diseases and certain malignancies [2, 3]. Prevention and early treatment of excess

body weight therefore serves as an important strategy to decrease the clinical and economic consequences of obesity. In line with this notion, weight loss of even 5–10% significantly improves impaired glucose tolerance in patients with T2D, decreases cardiovascular risk factors, lowers intra-abdominal and hepatic fat accumulation, improves β -cell function and enhances insulin sensitivity in liver, muscle and adipose tissue [4–6].

Conventional weight loss strategies built upon dietary interventions and exercise are failing to

tackle the global obesity pandemic [7, 8]. In addition, most historically used weight loss pharmacotherapies display an unfavourable imbalance between efficacy and safety. For example, several quite effective anti-obesity drugs such as fenfluramine/phentermine ('Fen-Phen') or rimonabant have been withdrawn from the market due to unacceptable adverse effects [9, 10]. Currently approved drugs for weight management, such as the gastric and pancreatic lipase inhibitor orlistat [11, 12], the serotonin receptor agonist lorcaserin [13–15] or the combination of the opioid antagonist naltrexone with the antidepressant bupropion [16–18], cause only moderate weight reductions. So far, the best weight-lowering effect by pharmacotherapies (approximately 7% body weight loss from baseline) is achieved by the injectable glucagon-like peptide-1 mimetic Saxenda® (liraglutide, 3 mg) [19], which is discussed later in this review.

Currently, the most effective anti-obesity therapy is a group of bariatric surgeries, including Roux-en-Y gastric bypass (RYGB), vertical sleeve gastrectomy (VSG) and biliopancreatic diversion. In contrast to the existing pharmacotherapies, bariatric surgery causes profound and sustained weight loss of 13–27% in severely obese patients (BMI > 35–40 kg m⁻²), with follow-up for as many as 15 years [20]. It can further ameliorate the majority of obesity-related comorbidities, including a full remission from T2D in approximately 80% of the patients [21–23]. The antidiabetic mechanisms are weight independent, which has prompted considerations of applying these surgeries to T2D patients with only mild (stage 1) obesity (BMI 30–35 kg m⁻²) [24]. Initial studies where RYGB surgery was applied to diabetic patients with stage 1 obesity indeed revealed significant but inconsistent remission rates between 25% and 88% [25, 26].

Despite the advantages of bariatric surgery, the highly invasive and irreversible nature of the surgeries, the underlying financial costs and the risk for severe adverse outcomes such as dumping syndrome, postprandial hyperinsulinaemic hypoglycaemia and the long-term risk of micronutrient deficiencies prevent the use of bariatric surgery as a widespread tool to tackle obesity and its comorbid sequelae.

Novel pharmacotherapies aim to mimic the complex and multi-target beneficial effects of bariatric

surgery on body weight and glycaemic control. Extensive research aims to uncover the molecular mechanisms that are driving the body weight and blood glucose lowering effects following the surgical interventions. It is now appreciated that the success of bariatric surgery is not solely due to mechanical aspects such as restriction in food intake and malabsorption but also involves physiological effects including altered gastrointestinal hormone secretion [27]. One of the most significant hormonal changes after bariatric surgery is the marked postprandial elevation of circulating glucagon-like peptide 1 (GLP-1), a powerful insulinotropic and anorectic hormone [28–30]. Although data from GLP-1 receptor (GLP-1R) knockout mice suggests that enhanced endogenous GLP-1 action is not the only driver for the metabolic benefits of bariatric surgery [31], it has been proposed that either more potent GLP-1 analogues or the combination of GLP-1 with other peptide hormones could serve as putative superior therapeutics for obesity and T2D. In line with this notion, the pharmacological inhibition of either GLP-1 or PYY after RYGB does not affect food intake. However, when both GLP-1 and PYY are blocked together, food intake is increased in patients with RYGB by as much as 20% [32]. Together, these data suggest that GLP-1, when acting in concert with other gut hormones, may play a causal role in the metabolic effects of bariatric surgery, and this has inspired the development of several GLP-1 analogues and GLP-1 combination therapies, as discussed in this review.

The endogenous GLP-1 system

GLP-1 is a member of the glucagon peptide family. Together with at least four other bioactive peptides, including GLP-2, glucagon, oxyntomodulin (OXM) and glicentin, it is derived from the proglucagon gene, which is expressed in the alpha-cells of the endocrine pancreas, the L cells of the intestine and neurons of the caudal brainstem and hypothalamus [33, 34]. The proglucagon mRNA is translated into a 180 amino acid precursor protein and post-translationally processed by cell type-specific prohormone convertase enzymes resulting in different organ-specific peptide profiles [35]. During the fasting and interprandial state, low levels of bioactive GLP-1(7-37) and GLP-1(7-36) amide are continuously secreted from the intestinal cells into the circulation. Following food intake, the secretion is rapidly increased and circulating GLP-1 levels rise by several fold [36]. The receptor for GLP-1, a class B G-protein coupled receptor, was originally cloned

from pancreatic β -cells [37]. The lack of specific antibodies against the GLP-1 receptor (GLP-1R) hampered several attempts to identify the cellular targets of GLP-1 action. Crossing *glp1r-cre* mice and fluorescent reporter strains resulted in an antibody-independent method for the identification of GLP-1R expressing organs [38]. This model revealed major expression sites of GLP-1R expression in pancreatic β - and δ -cells, vascular smooth muscle, cardiac atrium, gastric antrum/pylorus, enteric neurons, and vagal and dorsal root ganglia [38]. In the murine central nervous system, GLP-1R expression was evident in the circumventricular organs, amygdala, arcuate nucleus, paraventricular nucleus, and ventromedial hypothalamus and the ventrolateral medulla [38, 39].

The best-described target organs for GLP-1 mediated biological actions are the pancreas, the gastrointestinal and the central and peripheral nervous systems [34].

In pancreatic β -cells, GLP-1 receptor agonism stimulates glucose-dependent insulin secretion [29, 40–43]. GLP-1 further induces insulin biosynthesis [43] and promotes β -cell proliferation and survival in rodents [44–47]. GLP-1 also suppresses glucagon secretion from α -cells [48, 49]. Single-cell RNA sequencing revealed only very low levels of GLP-1R expression α -cells [50], suggesting that the inhibitory effect of GLP-1 on glucagon secretion occurs indirectly. One possible mechanism involves the binding of GLP-1 to its receptor on pancreatic δ -cells and the subsequent release of somatostatin, which in turn inhibits the release of glucagon from somatostatin receptor 2 (SSTR2) expressing α -cells. Evidence comes for instance from an experiment in isolated perfused rat pancreas, where co-infusion with a SSTR2 antagonist (PRL-2903) completely abolished the GLP-1-induced suppression of glucagon secretion [51]. GLP-1 may inhibit glucagon secretion through β -cell-derived products, such as insulin, GABA, zinc or amylin [52].

In the gastrointestinal system, GLP-1 receptor agonism exhibits a potent inhibitory effect on gastric emptying that attenuates the meal associated increase in blood glucose [53, 54].

Besides its glucometabolic effects, additional observations suggest that GLP-1 is also relevant for appetite regulation and weight maintenance. In rats, central administration of GLP-1 analogues

causes a dose-dependent, albeit short-lived reduction in food intake, independent of the presence of food in the stomach or gastric emptying [55, 56]. Similarly, peripheral administration of GLP-1 affects the regulation of feeding [57, 58]. These and other findings suggest a synergistic action of GLP-1 on both central and peripheral receptors in the regulation of satiety with the ultimate result of promoting weight loss. A more recent study investigated the GLP-1 analogue liraglutide in mice deficient in GLP-1R expression in either the vagal afferent/effect nerves or the central nervous system [59]. In this study, deletion of GLP-1R signalling in the central nervous system (CNS) ablates the action of the peripherally administered liraglutide on food intake and body weight, whereas deletion of GLP-1R signalling in the peripheral nervous system does not.

Additional GLP-1-mediated metabolic effects include the inhibition of hepatic gluconeogenesis and subsequent glucose output, an effect potentially mediated via GLP-1's ability to decrease glucagon secretion [60–62]. In the skeletal muscle, GLP-1 enhances glucose uptake [63] and glycogenesis [64].

All of these findings support GLP-1-based therapies for the effective treatment of obesity and T2D. However, the clinical applicability of native GLP-1 as an antidiabetic and anti-obesity therapy is limited by its short circulating half-life of 1–2 minutes in humans, which results from its deactivation by endopeptidase dipeptidyl peptidase-4 (DPP-4) and neutral endopeptidase 24.11 (NEP 24.11), also known as neprilysin [65–68]. Pharmacological inhibitors of DPP-4 have been developed with the aim to enhance the biological activity of endogenous GLP-1 [69]. Since 2006, several DPP-4 inhibitors including sitagliptin, saxagliptin and linagliptin have been approved for clinical use. However, when administered as monotherapies, DPP-4 inhibitors are weight neutral. Moreover, glycated haemoglobin A1C (HbA1C), a surrogate measure that reflects glycaemic exposure over the erythrocyte lifetime and current gold standard in assessment of metabolic control [70], is only modestly decreased (typically between 0.5% and 1%) after DPP-4 inhibitor treatment [71]. This suggests that supraphysiological levels of active GLP-1 are required to achieve a body weight-lowering effect and further improvements in glycaemic control. Pharmacological GLP-1 agonists can overcome this limitation. They are

chemically modified in order to enhance the stability and optimize pharmacokinetics to achieve a prolonged half-life compared to the endogenous GLP-1. This maximizes efficacy at low concentrations reduces the dosing frequency to improve patient convenience.

From gila monster venom to GLP-1 analogues

In 1992, a systematic investigation of the composition of the salivary secretions from the Gila monster (*Heloderma suspectum*) by John Eng and colleagues revealed a 39-amino acid peptide designated as exendin-4. Exendin-4 only shares 53% sequence homology with bioactive GLP-1(7-36) and is therefore considered a GLP-1 paralogue (Fig. 1), as opposed to a synthetic analogue of the type discussed throughout this review. Similar to GLP-1, exendin-4 is a α -helical peptide that interacts with the GLP-1 receptor, albeit with a much higher binding affinity [72]. The enhanced stability of exendin-4 results from a Leu21–Ser39 span, which builds a compact tertiary structure ('Trp-cage') that protects Trp25, Leu26 and Lys27 from aqueous solvent exposure and supports stabilization of secondary structure upon receptor binding [73, 74]. The receptor binding affinity of exendin-4 binding is further enhanced by a nine-residue extension at the C-terminal extension (CEX) of exendin-4 [75], Fig. 1.

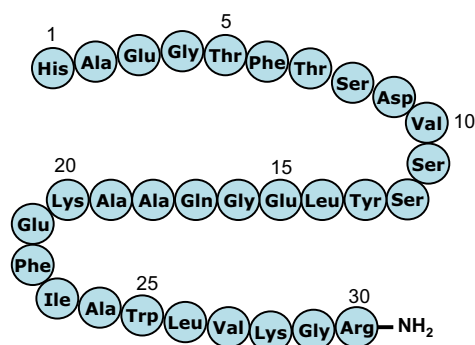
The discovery of exendin-4 has led to its experimental and clinical evaluation as an antidiabetic agent. In 2005, the first synthetic version of exendin-4, *exenatide BDI* (*Byetta*TM from *Amylin*,

now BMS), was approved for distribution and broad patient use. It has a prolonged half-life of 2.4 h, which results from the chemical benefits of the C-terminal Trp-cage and an additional alanine to glycine exchange at position two of the peptide, which increases resistance to DPP-4 mediated degradation [76].

The efficacy and safety of exenatide BDI have been evaluated in the AMIGO phase III clinical trials, where metformin, sulfonylurea or a combination of metformin and sulfonylurea at maximal effective doses were combined with either 5 μ g or 10 μ g of exenatide or placebo treatment. All studies were multicentre, randomized and triple blinded studies that enrolled more than 1.400 T2D patients with inadequate glycaemic control by metformin, sulphonylurea or the combination thereof. As a primary outcome, the addition of exenatide to any of the three conventional treatments resulted in a more significant reduction of HbA1C from baseline to week 30 compared to the matching placebo groups [77, 78]. The most common side effect of exenatide, nausea, was dose dependent and resulted in a dropout rate in the study of 1.8–4.0% [79]. Along with the improvements in glycaemic control, exenatide treatment caused a significant but moderate body weight reduction following 26 weeks of treatment with 10 μ g exenatide twice daily [80].

Lixisenatide (*Lyxumia*TM/*Adlyxin*TM, *Sanofi/Zealand*) is a second synthetic analogue of exendin-4, which has been modified by extending the C terminus of native exendin-4 to possess 6 lysine

GLP-1



Exendin-4

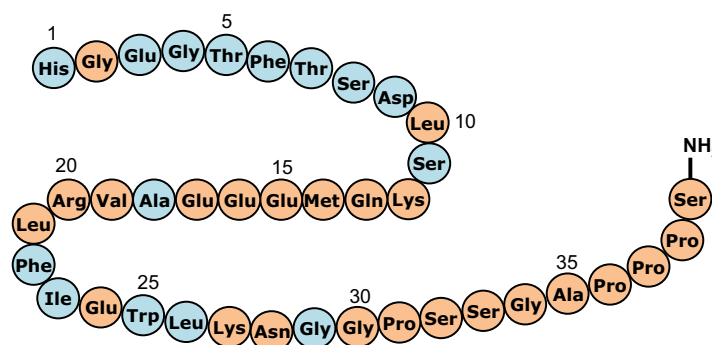


Fig. 1 Comparison of amino acid sequences for native human GLP-1 (left sequence) and exendin-4 (right sequence), which provide the basis for human GLP-1 analogues (*liraglutide*, *semaglutide*, *dulaglutide* and *albiglutide*) and exendin-4 derivatives (*exenatide*, *lixisenatide*).

Table 1 Head-to-head comparison of different types of glucagon-like peptide 1 receptor agonists

Clinical trial program	Comparator 1	Comparator 2	Background therapy	HbA1c reduction (Comparator 1 vs. 2)	Body weight loss (Comparator 1 vs. 2)	Refs.
GetGoal-X	Lixisenatide (20 µg once daily)	Exenatide BDI (10 µg twice daily)	Metformin	-0.80% vs. -0.96% (95% CI 0.033 to 0.297)	-2.96 kg vs. -3.98 kg (95% CI 0.45 to 1.58)	[82]
DURATION-1	Exenatide LAR (2 mg once weekly)	Exenatide BDI (10 µg twice daily)	Naive, or one or more oral antidiabetics	-1.9% vs. -1.5% (<i>P</i> -value < 0.0023)	-3.7 kg vs. -3.6 kg (<i>P</i> -value = 0.89)	[84]
LEAD-6	Liraglutide (1.8 mg once daily)	Exenatide BDI (10 µg twice daily)	Metformin, Sulfonylurea or both	-1.12% vs. -0.79% (<i>P</i> -value < 0.0001)	-3.24 kg vs. -2.84 kg (<i>P</i> -value = 0.22)	[87]
DURATION-6	Liraglutide (1.8 mg once daily)	Exenatide LAR (2 mg once weekly)	Metformin, Sulfonylurea or both or Metformin and pioglitazone	-1.48% vs. -1.28% (<i>P</i> -value < 0.02)	-3.57 kg vs. -2.68 kg (<i>P</i> -value < 0.0005)	[88]
HARMONY-7	Albiglutide (50 mg once weekly)	Liraglutide (1.8 mg once daily)	Metformin, pioglitazone, sulfonylurea or any combination thereof	-0.78% vs. -0.99% (<i>P</i> -value < 0.0846)	-0.64 kg vs. -2.16 kg (<i>P</i> -value < 0.001)	[91]
AWARD-6	Liraglutide (1.8 mg once daily)	Dulaglutide (1.5 mg once weekly)	Metformin	-1.36% vs. -1.42% (<i>P</i> -value < 0.0001)	-3.61 kg vs. -2.90 kg (<i>P</i> -value = 0.011)	[92]
SUSTAIN-7	Semaglutide (1.0 mg once weekly)	Dulaglutide (1.5 mg once weekly)	Metformin	-1.8% vs. -1.4% (<i>P</i> -value < 0.0001)	-6.5 kg vs. 3.0 kg (<i>P</i> -value < 0.0001)	[95]

residues with deletion of one C-terminal proline. The additional chemical modification slightly increased its half-life to 3–4 h allowing for once-daily subcutaneous administration, and, more importantly, it results in a four times more potent GLP-1 receptor binding [81]. Clinical efficacy of lixisenatide compared to exenatide BDI has been assessed in the 24-week GetGoal-X trial (Table 1), a randomized open label actively controlled study in T2D patients that were inadequately controlled by metformin therapy [82]. In this study, add-on lixisenatide (20 µg) demonstrated noninferior improvements in HbA1c, with slightly lower mean weight loss, but better gastrointestinal tolerability and lower incidence of hypoglycaemia compared

with twice-daily exenatide (10 µg). Lixisenatide was approved by the European Commission in 2013 and received FDA approval in 2016.

Despite the stabilization against DPP-4 mediated degradation, the half-life of GLP-1 analogues is still very short, due to its rapid renal clearance. One strategy to increase the plasma half-life was the development of slow release preparations, such as *exenatide LAR* (*Bydureon™*, *Amylin*, now *BMS*). In this preparation, exenatide is persistently and slowly released from poly(D,L-lactide-co-glycolide) forming microspheres [83]. This strategy increases the median plasma half-life from a few hours to 2 weeks. Approved in 2011, exenatide

LAR represents the first registered, once-weekly injectable drug against hyperglycaemia. The DURATION-1 clinical trial (Table 1) compared the once-weekly exenatide LAR (2 mg) with the twice-daily exenatide BDI (10 µg). After 30 weeks of treatment, the once-weekly formulation resulted in a significantly greater reduction of the HbA1C compared to the twice-daily formulation, whilst the body weight reduction remained similar between both groups [84].

Other strategies to improve peptide pharmacokinetics favoured the conjugation of GLP-1 analogues to long-chain fatty acids in order to achieve enhanced albumin binding and to hinder renal clearance. One example is *liraglutide* (*Victoza™* or *Saxenda™* Novo Nordisk). Liraglutide lacks the alanine to glycine exchange as seen in exendin-4 based GLP-1 analogues. Instead, an arginine residue replaces a lysine residue at position 28 and an additional glycine at position 31. Another lysine at position 20 is conjugated to a C16 palmitic acid via a gamma, glutamic acid spacer [85].

These chemical modifications lead to a self-association of the peptide into a heptameric structure, which delays the absorption from the injection site. In the bloodstream, extensive binding to albumin reduces its susceptibility to DPP-4 and NEP mediated cleavage, resulting in significant reduction in renal clearance. Liraglutide has a plasma half-life of 13 h [86]. In the LEAD-6 clinical trial (Table 1), a head-to-head comparison of once-daily liraglutide (1.8 mg) and twice-daily exenatide BDI (10 µg) added to a background treatment of metformin, sulphonylurea or a combination of both, liraglutide demonstrated a statistically significantly greater decrease in haemoglobin HbA1C than exenatide BDI [87]. Similarly, in the DURATION-6 trial, once-weekly exenatide LAR resulted in improvements in glycaemic control, with greater reductions as achieved with daily liraglutide [88], (Table 1). Liraglutide is now prescribed under two different brand names. *Victoza™* (FDA approval in 2010) is available in 1.2 mg and 1.8 mg doses and leads to an average HbA1c reduction of approximately 1.6% [89]. It is marketed for the treatment of type 2 diabetes but has only a subtle effect on body weight at these concentrations. *Saxenda™* (FDA approval in 2017) comes in a 3 mg dose and has been FDA approved for the treatment of obesity. In a clinical trial, *Saxenda™* resulted in average body weight loss of 8.5 kg over the course of the 56-

week study with mild or moderate nausea and diarrhoea being the most reported side effect [19].

A similar strategy to prolong the half-life of a peptide is its direct conjugation to recombinant albumin. In *albiglutide* (*Tanzeum™*, GlaxoSmithKline), two copies of the GLP-1(7-37) peptide are fused as a tandem repeat to the N terminus of recombinant albumin. A single alanine to glycine exchange at the DPP-4 cleavage site increased resistance to DPP-4-mediated cleavage. Albiglutide has a half-life of 6–8 days [90] and is administered once weekly at doses of 30–50 mg. However, as shown by the HARMONY-7 trial (Table 1), the average HbA1c reduction and weight-lowering effects of albiglutide were less when compared to liraglutide, thus not meeting the noninferiority criteria [91]. In August 2017 and only 3 years after its FDA approval, GlaxoSmithKline announced that albiglutide will be withdrawn from market by July 2018 for economic reasons.

Other recombinant GLP-1 fusion peptides have been developed. *Dulaglutide* (*Trulicity™*, Eli Lilly and Company, FDA approval in 2014) is a long-acting GLP-1 analogue in which a GLP-1(7-37) analogue has been covalently linked to each Fc arm of human immunoglobulin G4 (IgG4) to form a dimeric agonist, with the goal to prolong plasma circulation. Additional amino acid substitutions were made to increase resistance to DPP-4-mediated clearance (alanine to glycine at position 2). An exchange of Gly16 with Glu enhanced the secondary structure and potency of the peptide and an Arg30 to Gly exchange further enhances stability [85]. Overall this led to an increased half-life of dulaglutide of approximately 4 days. In the AWARD-6 clinical trial (Table 1), dulaglutide (1.5 mg once a week) met the predefined noninferiority criteria by causing a significantly greater reduction of HbA1c compared to liraglutide (1.8 mg once daily). However, weight reduction was significantly greater in the liraglutide treatment compared to the dulaglutide group, whilst the adverse side effects were comparable [92].

At present, liraglutide seems to be one of the most effective antiglycaemic and weight-lowering GLP-1 analogues. In December 2017, a next-generation liraglutide variant, named *semaglutide* (*Ozempic™*, NovoNordisk), was approved for commercial distribution. This chemically optimized version of liraglutide includes two modifications. A glycine in position 2 is replaced by the non-natural amino

acid aminoisobutyric acid (Aib) to increase resistance to degradation by DPP-4 and other serine proteases. The C16 fatty acid side chain conjugated to lysine at position 20, as present in liraglutide, has been exchanged with a dicarboxylic-stearic acid (C18:0) and a lengthier molecular spacer [85]. Both modifications further increase the half-life of the peptide in humans to 165 h [93]. Further modifications relative to endogenous GLP-1 are the lysine to arginine exchange at position 28 and the addition of a glycine at position 31.

The efficacy of once-weekly semaglutide has been assessed in clinical trials. In the SUSTAIN-1 trial, 30 weeks of a once per week semaglutide monotherapy resulted in a significant reduction of HbA1c of -1.43% (0.5 mg) and -1.53% (1.0 mg) compared to the placebo group. Simultaneously, in these T2D patients, treatment with semaglutide was associated with a significant weight loss -3.73 kg (0.5 mg semaglutide group) and -4.53 kg (1.0 mg group) compared to placebo [94]. In an additional head-to-head clinical trial (SUSTAIN-7, Table 1), semaglutide was superior to dulaglutide in improving glycaemic control and reducing body weight [95]. Other clinical trials are currently investigating oral vs. subcutaneous administration routes of semaglutide for the treatment of T2D.

When comparing all head-to head clinical trials, daily liraglutide, particularly when used at the highest doses, still appears to be the best, verified HbA1C and weight reduction pharmacotherapy [96]. Liraglutide remains the clinical standard for future advances, of which daily semaglutide therapy has been purported, but has yet to be peer-reviewed to deliver superior outcomes. Whilst the GLP-1 analogue field is continuously growing, there is still a need for optimization. At present, gastrointestinal side effects such as nausea and gastrointestinal discomfort limit the tolerability of GLP-1 analogues and their applicability at higher and maximal efficient doses. In view of these limitations and the still unprecedented benefits of bariatric surgeries, it was hypothesized that combining GLP-1 with other insulinotropic and/or anorectic peptide hormones could result in an enhanced efficacy and reduction of the dose-limiting toxicities. Intense efforts have been made to develop GLP-1 based combination therapies, as discussed further below.

GLP-1-based combination therapies

Previous studies have investigated GLP-1 analogues in combination with other weight-lowering drugs, such as PYY (3-36) [97–101], salmon calcitonin [102], leptin [103] or with an MC4R agonist (setmelanotide, RM-493) [104]. Although most studies demonstrated additive or synergistic effects of the drug combinations, the clinical application of these combinations is often challenging due to the different pharmacokinetic and pharmacodynamic profiles of the constituents, and the risk of unwanted drug–drug interactions. More recently, efforts have been made to combine two or more peptide hormones into one functional molecule, exerting only one pharmacokinetic profile and one targeted site of dual action, ideally resulting in a synergistic or complementary pharmacological action. The relative potency at each hormone receptor (either balanced or preferential) can be used to leverage efficacy and potency relative to unwanted dose-dependent side effects. Two different strategies have been used for the development of GLP-1-based unimolecular therapies: (A) fusion molecules, where GLP-1 is appended with another mono-agonist to form a bivalent molecule, and (B) hybrid molecules with comparable size to the native peptides [105]. The resulting molecules are summarized below.

GLP-1/Glucagon

Originally, glucagon was discovered as a peptide hormone with counter-regulatory effects to insulin [106]. Glucagon is secreted from pancreatic α -cells in response to hypoglycaemia and stimulates glycogenolysis and gluconeogenesis in the liver [85]. Patients with glucagonoma, a rare malignant tumour of the pancreatic α -cells, experience hyperglycaemia and as a result exhibit diabetes-like symptoms such as severe hyperglycaemia [107]. In contrast, postprandial glucose-mediated inhibition of glucagon secretion is impaired in patients with diabetes [108, 109]. Later studies demonstrated that blocking glucagon action through application of glucagon receptor antagonists or blocking antibodies significantly lowered fasting and postprandial glucose levels in different laboratory animal species [110–112], healthy subjects and diabetic patients [113, 114]. Apart from its hyperglycaemic actions, glucagon has several beneficial effects on energy and lipid metabolism (Fig. 2). For instance, administration of glucagon lowers circulating levels of cholesterol in multiple species [115–118]

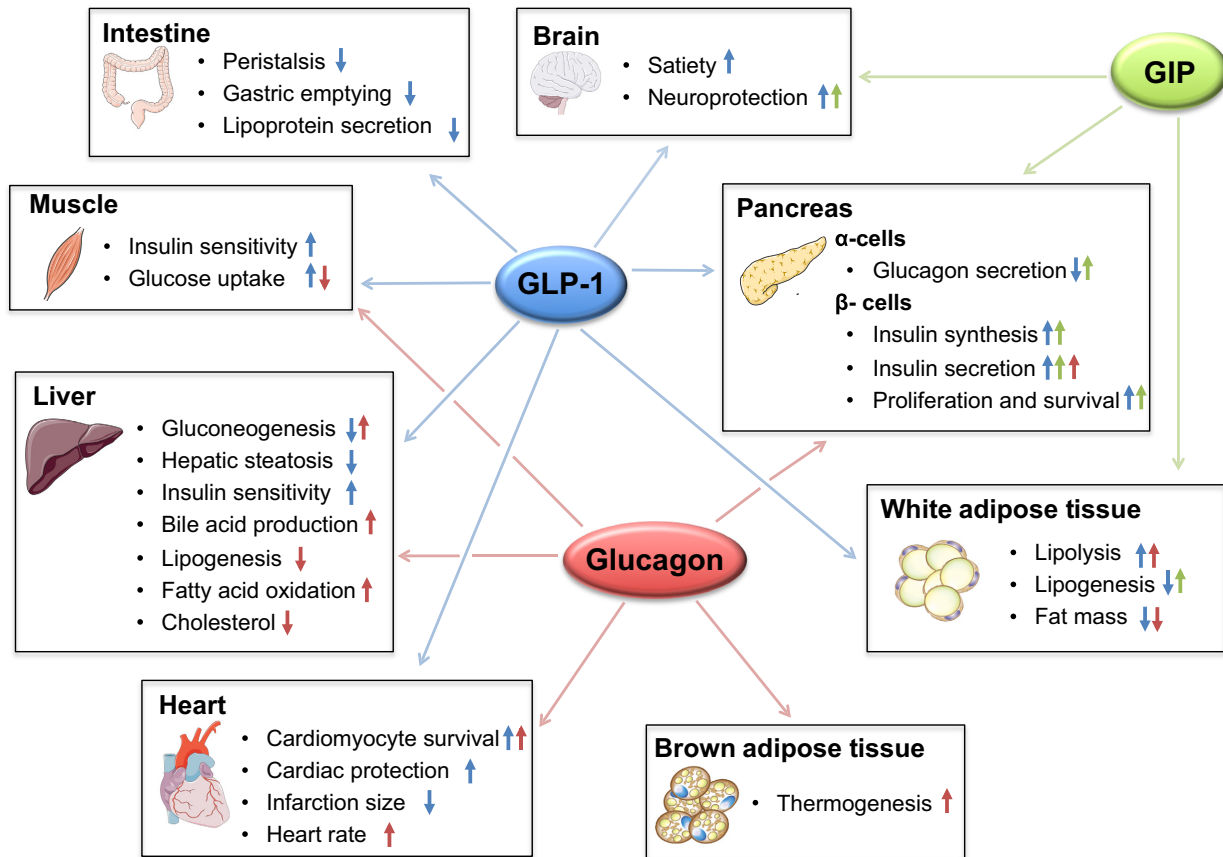


Fig. 2 Effects of GLP-1 (blue arrows), glucagon (red arrows) and GIP (green arrows) on energy metabolism in key metabolic tissues. Small arrows in boxes pointing upwards indicate an increase or improvement of the respective metabolic function, whilst arrows pointing downwards indicate a decrease.

and affects lipid metabolism through the inhibition of lipogenesis and stimulation of lipolysis [119–121]. In addition, glucagon is secreted during meals and acts in the central nervous system as a satiety signal to reduce food intake in humans [122–124] and rodents [125, 126]. Glucagon also stimulates energy expenditure and thermogenesis [127, 128], likely through the activation of brown adipose tissue (BAT) [128–130] and other, BAT-independent pathways [131]. The energy expenditure-stimulatory, hypolipidaemic and satiety effects suggest glucagon as an attractive therapy against obesity. Despite the plethora of positive effects, the acute hyperglycaemic effect of glucagon argued against the pharmacological application of glucagon as an anti-obesity drug [52].

Stemming from the same precursor protein, glucagon and GLP-1 exhibit considerable amino acid

similarity. Like the peptides, the GLP-1 and glucagon receptors are closely related, with an overall sequence homology of 58% [37, 132]. Structure–function analyses using truncated GLP-1 and glucagon peptides revealed specific residues throughout the length of the peptide that are important for receptor binding and activation. In 1994, Hjorth *et al.* [133] investigated a series of glucagon/GLP-1 chimeric peptides for their ability to bind and activate both receptors. The study revealed that residues located at the opposite ends of both peptides determine the receptor selectivity. A chimera containing N-terminal residues of glucagon and C-terminal residues of GLP-1 had high affinity for both receptors, but has not been tested *in vivo*.

In 2009, the research groups of Richard DiMarchi and Matthias Tschöp engineered a more complex

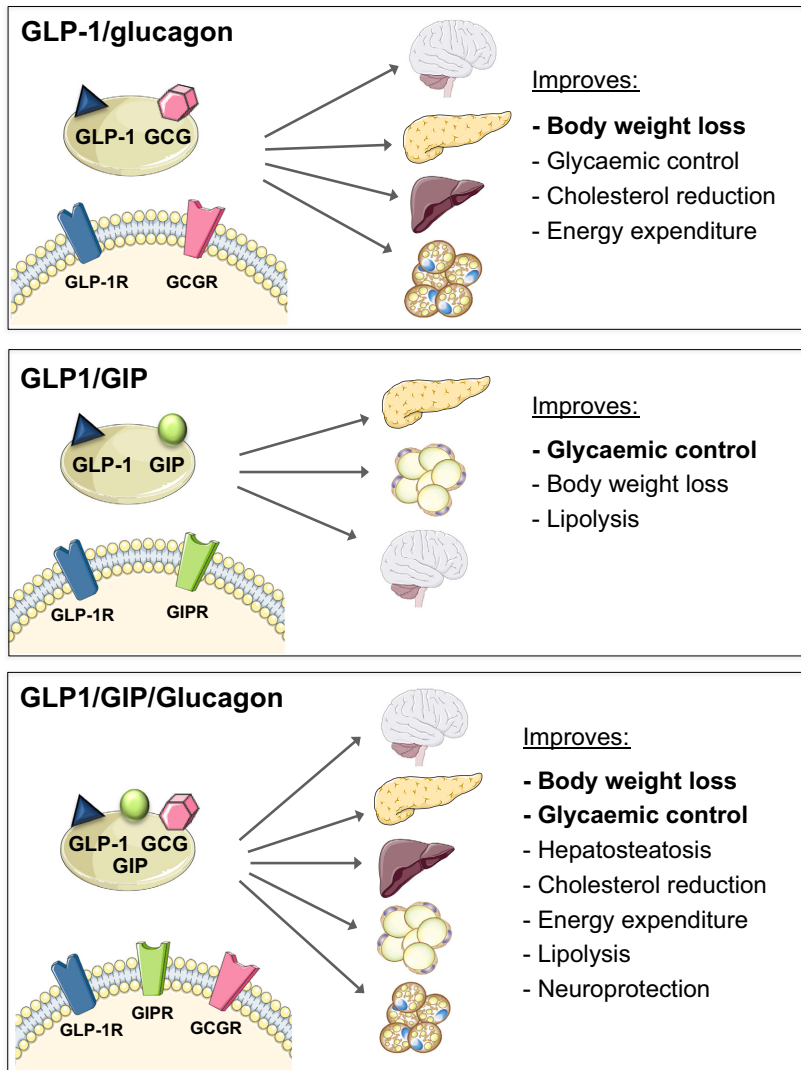


Fig. 3 Effects, working principles and target tissues of dual agonists GLP-1/ glucagon (upper panel) and GLP-1/ GIP (middle panel), and triple agonist GLP-1/ GIP/ glucagon (lower panel). The most predominant metabolic effects are indicated in bold letters.

dual GLP-1R/GCGR agonist by modifying the native glucagon sequence [134]. The authors hypothesized that dual agonism at both receptors would synergistically lower body weight by reducing food intake and stimulating energy expenditure, whilst the insulinotropic actions of GLP-1 would counter the hyperglycaemic liability of glucagon (Fig. 3). Combined agonism was achieved by a stepwise introduction of GLP-1 residues into the glucagon backbone to bolster GLP-1R activity.

GCGR activity was further enhanced by introduction of a lactam bridge between the glutamic acid at position 16 and the lysine at position 20, which stabilizes the alpha helix required for GCGR activation. An aminoisobutyric (Aib) acid at position 2 increased resistance to DPP-4 degradation, and a 40-kDa PEG attached via the cysteine at position 24 enhanced the pharmacokinetics of the molecule [85]. The result was a soluble and chemically stable glucagon-based peptide with nearly balanced

activity at both the GLP-1R and GCGR, and only slightly diminished potency compared to the natural ligands [134].

In DIO mice, a single high-dose injection of the PEGylated co-agonist (325 nmol kg⁻¹) induced a drop in body weight of 26% over the course of one week, primarily through the loss of fat mass, with an observed decrease in food intake. Longer-term treatment of DIO mice with lower doses (70 nmol kg⁻¹) of the same GLP-1R/GCGR agonist resulted in a weight loss comparable to the acute high-dose study. Notably, weight loss was associated with increased energy expenditure and thermogenesis, in line with glucagon's thermogenic capabilities, whilst no differences were observed in food intake or locomotor activity [134]. The importance of the glucagon moiety for the body weight loss was demonstrated in mice lacking the GLP-1R, in which the dual agonist maintained a significant weight-lowering capacity. Importantly, the GLP-1R KO mice did not show the previously observed benefits in glucose tolerance, underlining the importance of GLP-1 agonism to regulate glucose metabolism [134]. In addition to these findings, GLP-1R/GCGR receptor dual agonism provided benefits in lipid metabolism, normalization of liver lipid contents [134] and restored leptin sensitivity in DIO mice chronically maintained on a 58% HFD [135]. The beneficial effects of single molecule GLP-1R/GCGR co-agonism were also demonstrated in Lep^{ob/ob} mice, where the co-agonist enhanced glucose-stimulated insulin secretion and improved glucose tolerance [136].

Recently, the efficacy of a similar dual agonist of the GLP-1 and glucagon receptors (MEDI0382) was tested in rodents and cynomolgus monkeys. When compared to matched doses of liraglutide, both compounds reduced blood glucose to similar extents. The key differentiator from liraglutide was the superior weight loss in both species [137]. MEDI0382 has entered phase II trials, in which patients with controlled T2D received a once-daily subcutaneous injection of the dual agonist (300 µg for 22 days or 200 µg for 41 days) or a placebo treatment. MEDI0382 treatment resulted in a significant reduction of the glucose area under curve (AUC) following a mixed meal tolerance test. The bodyweight reduction was significantly greater with MEDI0382 than with placebo, suggesting its potential as a disease-modifying therapy for T2D [138].

Simultaneously to the development of the GLP-1/glucagon receptor co-agonist by Day *et al.*, the research group of Pocai *et al.* [139] developed a oxyntomodulin (OXM) analogue termed 'Dual AG'. Oxyntomodulin is a peptide hormone derived from proglucagon cleavage by PC1. Oxyntomodulin binds to both the GLP-1 and glucagon receptors, albeit with 10- to 100-fold reduction in potency compared to the native hormones [140–143]. The OXM analogue 'Dual AG' includes an amino acid exchange at position 2, which increases resistance to DPP-4 cleavage. A cholesterol moiety is conjugated via a cysteine side chain at the C terminus resulting in longer plasma retention. Daily subcutaneous injections of dual AG in diet-induced obese (DIO) mice for 2 weeks lowered body weight by 25%, primarily through the loss of fat mass and a fractional decrease in food intake [139]. In addition, treatment with dual AG also improved glucose tolerance, reduced plasma cholesterol and triglycerides and decreased hepatic steatosis [139]. Receptor knockout studies show reduced efficacy when either GLP-1R or GCGR is knocked out, indicating that both GLP-1R and GCGR agonism contribute to the metabolic actions of dual AG [139].

Based on these observations, GLP-1R/GCGR dual agonists may become effective metabolic therapies. Several pharmaceutical companies are developing GLP-1R/GCGR dual agonists to treat diabetes and obesity, and many of these potential therapeutics have progressed to clinical trials [144].

GLP-1/Amylin

Amylin (AYM), also known as islet amyloid polypeptide (IAPP), is cosecreted with insulin from the secretory granules of the β-cells. In contrast to insulin, which stimulates peripheral glucose uptake, amylin's glucose lowering effect is primarily mediated by suppressing pancreatic glucagon secretion. Amylin slows gastric emptying and promotes satiety, thus decreasing food intake [145] without causing food aversion [146]. Chronic amylin treatment elicits sustained weight loss in diet-induced obese rats and mice [147, 148].

Davalintide, a stable amylin analogue with 49% homology to the native hormone [149], was shown to cause significantly enhanced weight loss in rodent models when compared to rat amylin [149]. To further enhance the weight-lowering properties, the effects of incretin and amylin

classes of therapeutic hormones were combined by ligating davalintide and the GLP-1 analogue exenatide 1–28 into a single chemical entity using either a Gly-Gly-Gly (AC164204) or a β -Ala- β -Ala (AC164209) spacer, creating two new, so-called phybrids [150]. In obese and diabetic Lep^{ob/ob} mice, infusion with either phybrid reduced blood glucose and HbA1c levels to a similar extent as detected in the exenatide-treated group. However, both phybrids had a greater body weight-lowering effect compared to exenatide or davalintide monotherapies. In DIO rats, both phybrids caused a dose-dependent reduction of food intake and body weight [150]. The phybrid effect exceeded the exenatide or davalintide monotherapies, but was equal to the co-infusion of both single hormones. In another approach, the authors linked davalintide and exenatide 1–28 by a large intervening 40 kD PEG spacer [151]. This phybrid provides enhanced glycaemic control and weight loss in DIO rats and mice, along with a prolonged *in vivo* half-life of 27 h, compared to a side-chain PEGylated phybrid [151].

GLP-1/CCK

Cholecystokinin (CCK) and gastrin together constitute a family of structurally and functionally related peptide hormones. Both hormones share five terminal amino acids at the active carboxyl terminus (Gly-Trp-Met-Asp-Phe-NH₂). CCK is released from enteroendocrine I cells in the mucosal lining of the duodenum when fatty and amino acids leave the stomach and enter the small intestine [152]. There are several different forms of CCK, with the octapeptide CCK8 being the most abundant in the brain [152]. This peptide has been implicated in satiety, as acute administration of CCK8 reduces meal size in rodents, although this effect is counterbalanced by an increase in the number of meals, which mitigates the initial meal size reduction [152].

There are two CCK receptors, termed CCKA and CCKB, or more recently, CCK1 and CCK2. CCK1 is abundantly expressed in the brain areas mediating satiety, such as the solitary nucleus (NTS), area postrema (AP) and the dorsal medial hypothalamus (DMH). CCK1 mediates the inhibitory effects of CCK on food intake [152]. The CCK2 receptor, identical to the gastrin receptor, is also present in the CNS. Both gastrin and the C-terminal amidated form of CCK bind to this receptor [153]. Recently, the co-administration of CCK and a GLP-1R

analogue resulted in synergistic weight loss in rodents [154, 155], paving the way for a stable (pGlu-Gln)-CCK-8/exendin-4 hybrid in which the key amino acid sequences of the well-characterized, stable and specific CCK-8 and GLP-1 analogues (pGlu-Gln)-CCK-8 and exendin-4 were ligated through a (2-[2-aminoethoxy]ethoxy)acetic acid linker [156]. The fusion peptide demonstrated decreased energy intake and lowering of body weight in NIH Swiss mice fed a high-fat diet, with metabolic improvements that were not seen with a matched dose of exendin-4 alone [156]. Compared to the monotherapies, the conjugate also improved glucose tolerance and insulin sensitivity [156]. In a recent study, another fusion peptide (C2816) comprised of a stabilized GLP-1R agonist (AC3174) and a CCKR1-selective agonist (AC170222) exerted a superior reduction in body weight compared to co-administration of AC3174 and AC170222 in DIO mice [157].

GLP-1/Gastrin

The structural and functional similarity between CCK-8 and gastrin naturally suggested the combination of GLP-1R and gastrin. Gastrin is synthesized by the G cells in the stomach and duodenum, is released in response to meal ingestion and binds to the CCK2 receptor [153]. A dual agonist of the GLP-1 and CCK2 receptors, ZP3022, lowers body weight and improves glucose tolerance in male db/db mice [158]. ZP3022 also increases pancreatic β -cell mass, without increasing the number of pancreatic islets, whilst simultaneously increasing insulin levels in these mice [158]. The exact mechanism of action remains unknown, but it is speculated that GLP-1 action on β -cells, in combination with indirect gastrin action, is responsible for the observed pancreatic islet expansion. In a more chronic, 8-week study in ZDF rats, ZP3022 significantly reduced body weight and blood glucose and increased the pancreatic β -cell fraction compared to vehicle-treated controls [159], suggesting that this peptide has potential as an antidiabetic pharmacotherapy.

GLP-1/GIP

The glucagon backbone was used as a template to generate another hybrid peptide with dual agonism at the receptors for GLP-1 and glucose-dependent insulinotropic polypeptide (GIP), Fig. 3 [134]. GIP is another member of the glucagon peptide family. It is derived from a 153-amino acid proprotein

encoded by the GIP gene and is secreted from the intestinal K cells in response to a meal, and promotes insulin secretion in a glucose-dependent manner [160, 161]. Beyond its insulinotropic action, GIP also stimulates the release of glucagon under conditions of hypoglycaemia [162] (Fig. 2). Therefore, as a bifunctional hormone, it may be doubly capable of stabilizing blood glucose levels [163, 164]. Despite these clear glycaemic benefits, the application of GIP analogues as pharmacological targets against T2D has long been hindered by its suggested role as an obesogenic, lipogenic and adipogenic peptide in rodents and humans [165–169]. However, more recent studies show that overexpression of GIP as well as GIP agonism improved glucose metabolism in DIO mice, without detrimental effects on body weight [170, 171]. Similarly, transgenic pigs expressing a dominant-negative GIP receptor in pancreatic islets developed a diabetic phenotype without apparent changes in body weight [172]. In rats, central delivery of GIP even decreased the body weight compared to the vehicle-injected control animals [173].

Since both GLP-1 and GIP are insulinotropic, the combination of GLP-1R and GIPR agonism was hypothesized to result in additive or even synergistic effects on insulin secretion and glucose tolerance (Fig. 3). Moreover, the anorectic effect of GLP-1 could buffer the alleged obesogenic effect of GIP. Indeed, the combination of liraglutide and an acylated GIP was more potent at lowering blood glucose and stimulating insulin secretion in leptin-deficient *Lep^{ob/ob}* mice than the single compounds [174]. Similarly, in healthy human volunteers, co-infusions of synthetic GLP-1 and GIP analogues additively increased the insulinotropic action relative to the monotherapies [175]. Interestingly, in T2D patients, adding GIP to GLP-1 did not further enhance the insulinotropic activity of GLP-1 but antagonized the GLP-1 mediated suppression of glucagon [176].

Although GLP-1 and GIP only share 37% of their amino acid sequence, their receptor binding domains are very similar. Therefore, whilst designing dual agonists with affinity for both incretin receptors is possible, it is much more challenging than what was initially achieved with GLP and glucagon. Recently, a series of unimolecular GLP-1/GIP peptides has been developed to achieve potent and balanced co-agonism at both receptors with negligible cross-reactivity at the glucagon receptor [177]. In one example, amino acids were

introduced stepwise to the glucagon sequence to impart GLP-1R and GIPR activity. To extend its *in vivo* activity and plasma half-life, an Aib residue at position 2 increased resistance to DPP-4 degradation, and the nine amino acid CEX extension provided additional stability and aqueous solubility. To prevent unwanted GCGR activity, an additional Aib residue was incorporated at position 20, which partially stabilizes the secondary structure of the molecule and minimizes GCGR activity. Finally, a cysteine at position 24 or lysine at position 40 was included to serve as unique sites for subsequent conjugation to fatty acyl, or PEG polymers. Whilst both peptide versions demonstrated balanced receptor activities, acetylation at Lys40 resulted in slightly increased receptor potency and PEGylation at Cys24 diminished receptor potencies when compared to the natural peptide hormones. In DIO and leptin receptor-deficient *db/db* mice, the fatty acylated and PEGylated versions of the co-agonist resulted in superior antihyperglycaemic and insulinotropic efficacy, with profound body weight lowering relative to a pharmacokinetically matched GLP-1 mono-agonist, such as exendin-4 and liraglutide [177]. Notably, the apparent safety and insulinotropic efficacy of the acylated version of the unimolecular GLP-1/GIP co-agonist translated from rodent models of obesity to cynomolgus monkeys [177]. The same compound (formerly NNC0090-2746, now RG7697) was also tested in human patients with type 2 diabetes on a metformin background therapy using a dose comparable to liraglutide [178]. Daily subcutaneous injections of 1.8 mg of the co-agonist for 12 weeks decreased HbA1c by 0.96% and fasting glucose by 38.2% relative to placebo, and reduced body weight in an absolute sense by nearly 3% over the twelve-week trial [178]. Notably, RG7697 significantly decreased total cholesterol, compared to the placebo group, whereas liraglutide alone had no effect, suggesting an additional benefit of the dual GLP-1/GIP receptor agonist. Other independently derived GLP-1R/GIPR co-agonists are in development and are being investigated in preclinical and phase 2 clinical trials [144].

GLP-1/Glucagon/GIP

The success of the incretin co-agonists naturally led to the hypothesis that a triagonist, with agonism at both incretin hormone receptors and the glucagon receptor, would result in even more effective metabolic improvements (Fig. 3).

Optimally, the glycaemic benefits arising from both incretin hormones would oppose glucagon's diabetogenic actions, whilst the weight-lowering properties of GLP-1 and glucagon would synergistically suppress any potential obesogenic character residing in GIP agonism.

In 2013, three different peptides with triple agonism at the GLP-1R, GCGR and GIP-R were developed. The first example replaces the initial 11 N-terminal residues of OXM with D-Ala-GIP to generate a GIP-OXM peptide ([DA2]GIP-Oxm) [179]. A second triagonist, [DA2]GLP-1-glucagon ([DA2]GLP-1/GcG), was created as a fusion of key amino acid sequences from GLP-1, GIP, and glucagon [136]. The third triagonist, YAG-glucagon, was derived by manipulating the glucagon peptide sequence [180]. All three peptides stimulated cAMP production in GIP-R, GCGR and GLP-1R transfected cells to a comparable or lesser extent than the native peptides, demonstrating triple agonism *in vitro*. *In vivo*, all peptides significantly reduced glycaemia, whilst only [DA2]GIP-Oxm and [DA2]GLP-1/GcG significantly decreased body weight. Since both GLP-1R and GCGR agonism result in body weight loss, the inability of YAG-glucagon to lower body weight may be a result of unbalanced agonism towards the GIP receptor.

Similarly, in 2015, the research groups of Matthias Tschöp and Richard DiMarchi developed another novel triagonist, beginning with a validated GLP-1R/GIPR co-agonist and introducing amino acids known to confer GCGR agonism in a stepwise fashion [181]. Further modifications included an Aib at position 2 to increase resistance to DPP-4 degradation, a lysine at position 10 (Lys10) as an attachment site for a palmitic acid, and the CEX extension to improve the solubility of the peptide [181]. The triagonist displayed full GLP-1R agonism in pancreatic mouse β -cells (MIN6), full GIPR activity in mouse 3T3-L1 adipocytes, and full GCGR activity in rat hepatocytes [181]. In addition, compared to [DA2]GLP-1/GcG, this triagonist was at least 1000-fold more potent at all three receptors *in vitro* [181]. In DIO mice, daily treatment at 3 nmol kg⁻¹ of this triagonist lowered body weight by 26.6% over a 20-day period, compared to only 15.7% loss with a dose-matched GLP-1/GIP co-agonist. Body weight loss was mainly the result of loss in fat mass and not lean mass. The triagonist induced superior glycaemic control and reduction in hepatic lipid content, all greater than a matched dose of liraglutide [181]. These effects are not gender-specific, as similar

reductions in body weight and hepatic steatosis were observed in female DIO mice [181, 182]. Compared to [DA2]GLP-1/GcG, this triagonist induces body weight loss and metabolic improvements at much lower doses.

Chronic treatment with this triagonist in lean mice resulted in no reduction of body weight, lean mass or food intake [181], suggesting that the triagonist does not impair normal metabolism and only acts to improve metabolic dysregulation. Moreover, there were no instances of hypoglycaemia in either DIO or lean mice treated with the triagonist, demonstrating that the hypoglycaemic liability of glucagon receptor agonism is safely managed. The triagonist preserved pancreatic islet architecture in ZDF rats and db/db mice, suggesting that the triagonist has potential as both an anti-obesity and an antidiabetic therapy [181]. Meanwhile, several triagonist peptides have entered preclinical trials [144]. First results are published for the compound HM15211, developed by Hanmi Pharmaceuticals, a triple agonist based on a modified glucagon analogue with activity at all three receptors [183]. This triple agonist is modified with a human glycosylated Fc fragment to prolong the half-life. In rodent models, every other day treatment with HM15211 decreased body weight and glycaemia, whilst increasing energy expenditure to a significantly greater extent than a daily administration of liraglutide. In addition, HM15211 reduces hepatic steatosis and plasma cholesterol in a mouse model of NASH, indicating therapeutic potential beyond weight loss and glycaemic control [183]. HM15211 is currently being investigated in phase 1 clinical trials.

In summary, the triagonists developed so far have demonstrated unmatched preclinical efficacy in improving metabolic dysregulation that may recapitulate many benefits of bariatric surgeries. Moreover, based on the multi-organ receptor expression, the triagonists could have great potential to treat a number of other diseases. Indeed, first results have demonstrated that the triagonist HM15211 exerted neuroprotective effects against Parkinson's disease by reducing microglia activation [184] and is effective against NASH [183].

GLP-1-based nuclear hormone delivery

Similar to the peptide hormones reviewed to this point, certain nuclear hormones such as oestrogen, thyroid hormone (T₃) and dexamethasone are potent and beneficial modulators of energy

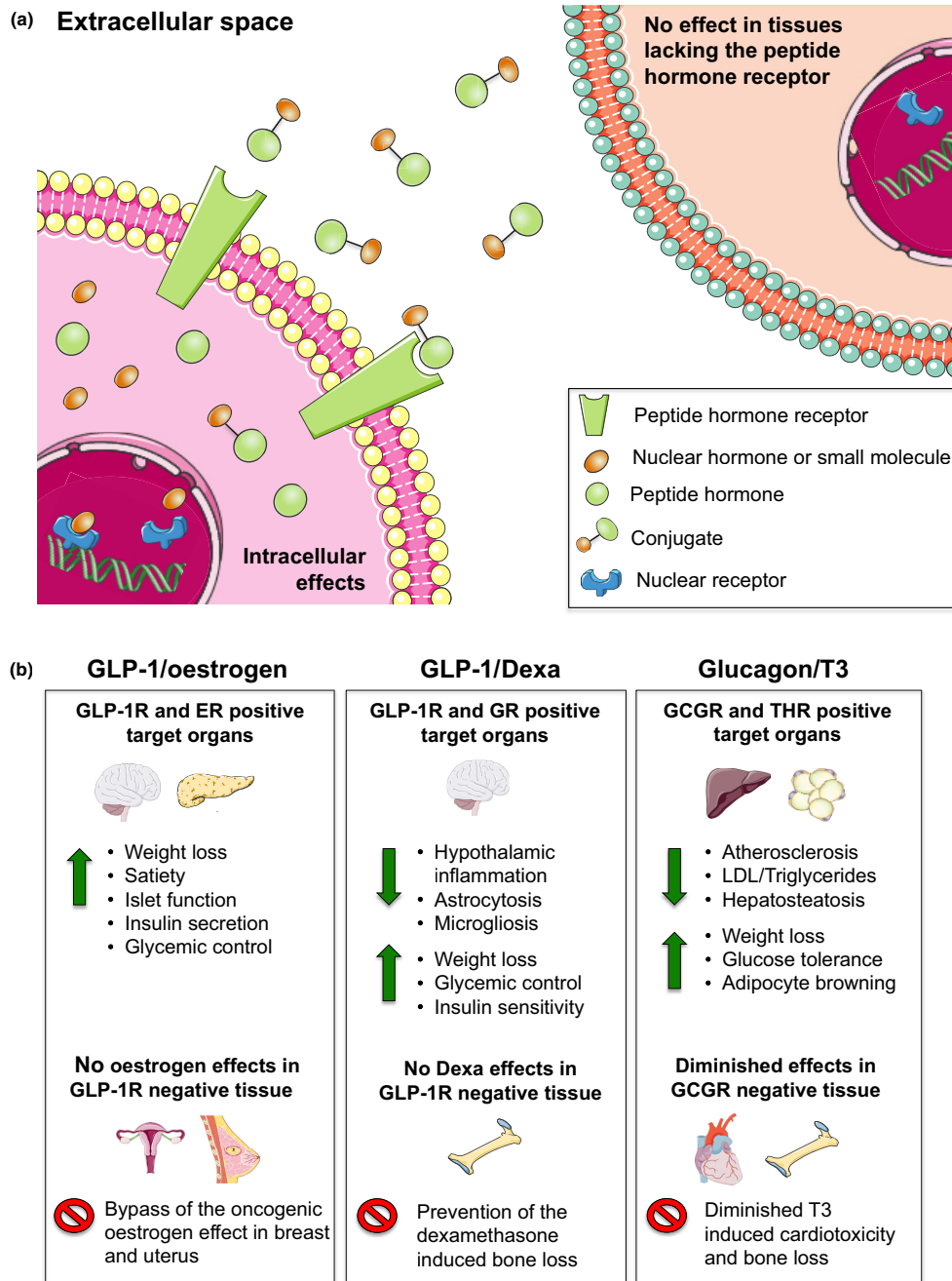


Fig. 4 (a) Schematic for the peptide hormone-mediated delivery of small molecules and nuclear hormones via receptor internalization. (b) Metabolic effects and major target organs of GLP-1/oestrogen, GLP-1/dexamethasone (GLP-1/Dexa), and glucagon/T3 hybrid molecules and bypass of established adverse side effects of oestrogen, dexamethasone or T3 by their targeted delivery to GLP-1 receptor (GLP1-R) or glucagon receptor (GCGR) expressing organs (lower boxes).

metabolism [185–188]. However, their medical utility is restricted due to notable unwanted side effects. A novel approach to avoid an impact on off-target tissues was to covalently link nuclear hormones to peptide hormones such as GLP-1 and glucagon. Peptide hormones promote their biological action via binding and activation of receptors located on the cell surface, followed by internalization of the ligand–receptor complex and activation of downstream signalling pathways. In the context of metabolic therapy, GLP-1 is an ideal nuclear hormone conjugation partner since GLP-1 targets mainly the endocrine pancreas and central nervous system, thus potentially delivering nuclear hormones preferentially to these tissues. So far GLP-1 has been conjugated to oestrogen [189] and dexamethasone [190] (Fig. 4). In addition, T₃ has been conjugated to glucagon [191] (Fig. 4). Conjugation to GLP-1 has resulted in targeted benefits of the respective nuclear hormones. Treatment of male and female DIO mice with a stable GLP-1/oestrogen conjugate induced synergistic weight loss and metabolic improvements, which were dependent on the presence of the GLP-1R in the central nervous system [189]. The oestrogen effect of the conjugate was limited to GLP-1R expressing tissues and did not cause any oestrogen-related gynaecological or tumour promoting effects in tumour-bearing mice, nor did it affect bone mineral density.

A conjugate of GLP-1 and dexamethasone utilizes the anti-inflammatory properties of dexamethasone to target the chronic, low-grade inflammation that is typically observed under conditions of obesity [192, 193]. Unaltered dexamethasone induces hyperglycaemia, hyperphagia and reduces bone density [190]. In mice, a GLP-1/dexamethasone conjugate at a dose of 100 nmol kg⁻¹ for 2 weeks reduced food intake and induced a 25% body weight loss, relative to baseline, predominantly a result of loss in fat mass. The metabolic benefits of the conjugate are due in part to an increase in energy expenditure, since the conjugate increases oxygen consumption, reduces the respiratory exchange ratio (RER) and induces greater body weight loss relative to pair-fed controls. In addition, the anti-inflammatory action of dexamethasone was apparent both in the hypothalamus and in plasma, where the conjugate reduced cytokine levels and other markers of inflammation. The lack of GLP-1 receptors in the liver precluded dexamethasone related effects on hepatic glucose output and hyperglycaemia. Nevertheless, the

conjugate improved glucose tolerance and increased glucose-stimulated insulin secretion, indicating positive glycaemic effects. In addition, the conjugate does not appear to affect bone density, as whole-body and spine bone mineral density were unaltered by treatment [190].

Another approach for targeted nuclear hormone delivery was the covalent binding of T₃ to glucagon. Since GCGR expression is largely restricted to the liver, this hybrid molecule was designed to accentuate the hepatic effects of T₃, which include clearing of circulating LDL via stimulation of reverse cholesterol transport and enhanced production of bile acids. Abnormally high cholesterol and dyslipidaemia are a major health concern, and dyslipidaemia is often associated with type 2 diabetes, coronary heart disease and nonalcoholic steatohepatitis (NASH) [194]. Most dyslipidaemia drugs, such as statins, lower cholesterol but do not affect body weight. It was thus hypothesized that glucagon-directed hepatic T₃ action would synergistically improve hepatic lipid and cholesterol metabolism and simultaneously counteract the hyperglycaemic actions of glucagon and that targeted delivery to glucagon receptor expressing cells would assure that T₃ does not reach and act on tissues such as the heart, skeletal muscle and bone to cause unwanted side effects such as cardiotoxicity.

Indeed, in DIO mice, the conjugate lowered blood glucose, improved glucose tolerance and dose dependently prevented the development of hyperglycaemia or glucose intolerance [191]. The glucagon/T₃ conjugate moderately lowered body weight by reducing food intake and increasing energy expenditure. Moreover, in various mouse models of dyslipidaemia, the glucagon/T₃ conjugate lowered total plasma cholesterol and decreased circulating and hepatic levels of LDL. These mice also display lowered hepatic cholesterol and a decrease in hepatocellular vacuolation. These effects were lost in global GCGR knockout mice and in liver-specific thyroid hormone receptor- β knockout mice [191], demonstrating the necessity of both the glucagon receptor and thyroid hormone action in the liver. The glucagon/T₃ conjugate was demonstrably safer than either of its individual components, with only limited T₃ effects in heart and bone. The full magnitude of the improvements in the therapeutic index will nevertheless require more extensive studies, extension of these initial reports from rodents to primates, followed by refinement of the

chemical structure to suit drug development with completion of preclinical safety studies supportive of translation to clinical study.

Conclusion

Combining the actions of multiple hormones into single molecular entities has resulted in multi-action agonists that display superior efficacy and safety when compared to the constituent monotherapies.

Multi-agonism results in enhanced activity through synergistic agonism at multiple receptors. Many of these multi-agonists have proven more effective than either the monotherapies or the co-injection of the hormones. This enhanced activity allows for lower dosing strategies, which decreases the risk of dose-dependent adverse effects. In addition, a multi-agonist approach can overcome inherent liabilities of individual hormones, by targeting nuclear hormones to specific tissues and providing counter-regulatory buffering activity.

The multi-agonist pharmacotherapies in this review constitute great translational potential and promising preclinical results are emerging. It is premature to conclude the magnitude of the pharmacology that might be achieved in human patients. However, it feels inevitable that within this broad set of agonists that function by multiple, differentiated mechanisms that a meaningful enhancement to the efficacy rendered by GLP-1 specific pharmacology should emerge through continued research and refinement of those entities that prove most effective.

Acknowledgement

The figures were made using material provided by Servier Medical Art (Servier), under the terms of the Creative Commons Attribution 3.0 Unported License.

Funding

This work was supported in part by funding to M H T from the Alexander von Humboldt Foundation, the Helmholtz Alliance ICAMED & the Helmholtz Initiative on Personalized Medicine iMed by Helmholtz Association and the Helmholtz cross-program topic 'Metabolic Dysfunction'. This work was further supported by grants from the German Research Foundation DFG-TS226/1-1, DFG-

TS226/3-1, SFB 1321/1, SFB TRR 152, European Research Council ERC AdG HypoFlam no. 695054 and the German Center for Diabetes Research (DZD e.V.).

Conflict of Interest

S.J.B., T.D.M. and K.S. declare that there are no conflict of interests that could be perceived as prejudicing the impartiality of this review. R.D.D. is current employee of Novo Nordisk. R.D.D. is a cofounder of Marcadia, a company that pioneered the discovery of glucagon mixed agonists. It was acquired by Roche and later Novo Nordisk. M.H.T. is a scientific advisor for Novo Nordisk and Erx Biotech.

References

- 1 Afshin A, Forouzanfar MH, Reitsma MB *et al.* Health effects of overweight and obesity in 195 countries over 25 years. *N Engl J Med* 2017; **377**: 13–27.
- 2 Fontaine KR, Redden DT, Wang C, Westfall AO, Allison DB. Years of life lost due to obesity. *JAMA* 2003; **289**: 187–93.
- 3 Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. *Nat Rev Cancer* 2004; **4**: 579–91.
- 4 Heymsfield SB, Wadden TA. Mechanisms, pathophysiology, and management of obesity. *N Engl J Med* 2017; **376**: 1492.
- 5 Magkos F, Fraterrigo G, Yoshino J *et al.* Effects of moderate and subsequent progressive weight loss on metabolic function and adipose tissue biology in humans with obesity. *Cell Metab* 2016; **23**: 591–601.
- 6 Wing RR, Lang W, Wadden TA *et al.* Benefits of modest weight loss in improving cardiovascular risk factors in overweight and obese individuals with type 2 diabetes. *Diabetes Care* 2011; **34**: 1481–6.
- 7 Bray GA. Lifestyle and pharmacological approaches to weight loss: efficacy and safety. *J Clin Endocrinol Metab* 2008; **93**: S81–8.
- 8 Kraschnewski JL, Boan J, Esposito J *et al.* Long-term weight loss maintenance in the United States. *Int J Obes (Lond)* 2010; **34**: 1644–54.
- 9 Centers for Disease C, Prevention. Cardiac valvulopathy associated with exposure to fenfluramine or dexfenfluramine: U.S. Department of Health and Human Services interim public health recommendations, November 1997. *MMWR Morb Mortal Wkly Rep* 1997; **46**: 1061–6.
- 10 Samat A, Tomlinson B, Taheri S, Thomas GN. Rimonabant for the treatment of obesity. *Recent Pat Cardiovasc Drug Discov* 2008; **3**: 187–93.
- 11 Drent ML, Larsson I, William-Olsson T *et al.* Orlistat (Ro 18-0647), a lipase inhibitor, in the treatment of human obesity: a multiple dose study. *Int J Obes Relat Metab Disord* 1995; **19**: 221–6.
- 12 Sahebkar A, Simental-Mendia LE, Reiner Z *et al.* Effect of orlistat on plasma lipids and body weight: a systematic review and meta-analysis of 33 randomized controlled trials. *Pharmacol Res* 2017; **122**: 53–65.

- 13 Smith SR, Weissman NJ, Anderson CM *et al.* Multicenter, placebo-controlled trial of lorcaserin for weight management. *N Engl J Med* 2010; **363**: 245–56.
- 14 Fidler MC, Sanchez M, Raether B *et al.* A one-year randomized trial of lorcaserin for weight loss in obese and overweight adults: the BLOSSOM trial. *J Clin Endocrinol Metab* 2011; **96**: 3067–77.
- 15 O'Neil PM, Smith SR, Weissman NJ *et al.* Randomized placebo-controlled clinical trial of lorcaserin for weight loss in type 2 diabetes mellitus: the BLOOM-DM study. *Obesity (Silver Spring)* 2012; **20**: 1426–36.
- 16 Greenway FL, Fujioka K, Plodkowski RA *et al.* Effect of naltrexone plus bupropion on weight loss in overweight and obese adults (COR-I): a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2010; **376**: 595–605.
- 17 Apovian CM, Aronne L, Rubino D *et al.* A randomized, phase 3 trial of naltrexone SR/bupropion SR on weight and obesity-related risk factors (COR-II). *Obesity (Silver Spring)* 2013; **21**: 935–43.
- 18 Hollander P, Gupta AK, Plodkowski R *et al.* Effects of naltrexone sustained-release/bupropion sustained-release combination therapy on body weight and glycemic parameters in overweight and obese patients with type 2 diabetes. *Diabetes Care* 2013; **36**: 4022–9.
- 19 Pi-Sunyer X, Astrup A, Fujioka K *et al.* A randomized, controlled trial of 3.0 mg of liraglutide in weight management. *N Engl J Med* 2015; **373**: 11–22.
- 20 Sjostrom L, Gummesson A, Sjostrom CD *et al.* Effects of bariatric surgery on cancer incidence in obese patients in Sweden (Swedish Obese Subjects Study): a prospective, controlled intervention trial. *Lancet Oncol* 2009; **10**: 653–62.
- 21 Adams TD, Davidson LE, Litwin SE *et al.* Weight and metabolic outcomes 12 years after gastric bypass. *N Engl J Med* 2017; **377**: 1143–55.
- 22 Carlsson LM, Peltonen M, Ahlin S *et al.* Bariatric surgery and prevention of type 2 diabetes in Swedish obese subjects. *N Engl J Med* 2012; **367**: 695–704.
- 23 Buchwald H, Estok R, Fahrenbach K *et al.* Weight and type 2 diabetes after bariatric surgery: systematic review and meta-analysis. *Am J Med* 2009; **122**: 248–56 e5.
- 24 Rubino F, Nathan DM, Eckel RH *et al.* Metabolic surgery in the treatment algorithm for type 2 diabetes: a joint statement by international diabetes organizations. *Surg Obes Relat Dis* 2016; **12**: 1144–62.
- 25 Cohen RV, Pinheiro JC, Schiavon CA, Salles JE, Wajchenberg BL, Cummings DE. Effects of gastric bypass surgery in patients with type 2 diabetes and only mild obesity. *Diabetes Care* 2012; **35**: 1420–8.
- 26 Scopinaro N, Adami GF, Papadia FS *et al.* Effects of gastric bypass on type 2 diabetes in patients with BMI 30 to 35. *Obes Surg* 2014; **24**: 1036–43.
- 27 Evers SS, Sandoval DA, Seeley RJ. The physiology and molecular underpinnings of the effects of bariatric surgery on obesity and diabetes. *Annu Rev Physiol* 2017; **79**: 313–34.
- 28 Naslund E, Backman L, Holst JJ, Theodorsson E, Hellstrom PM. Importance of small bowel peptides for the improved glucose metabolism 20 years after jejunoileal bypass for obesity. *Obes Surg* 1998; **8**: 253–60.
- 29 Kreymann B, Williams G, Ghatei MA, Bloom SR. Glucagon-like peptide-1 7-36: a physiological incretin in man. *Lancet* 1987; **2**: 1300–4.
- 30 Mojsos S, Weir GC, Habener JF. Insulinotropin: glucagon-like peptide I (7-37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. *J Clin Invest* 1987; **79**: 616–9.
- 31 Wilson-Perez HE, Chambers AP, Ryan KK *et al.* Vertical sleeve gastrectomy is effective in two genetic mouse models of glucagon-like Peptide 1 receptor deficiency. *Diabetes* 2013; **62**: 2380–5.
- 32 Svane MS, Jorgensen NB, Bojsen-Moller KN *et al.* Peptide YY and glucagon-like peptide-1 contribute to decreased food intake after Roux-en-Y gastric bypass surgery. *Int J Obes (Lond)* 2016; **40**: 1699–706.
- 33 Mojsos S, Heinrich G, Wilson IB, Ravazzola M, Orci L, Habener JF. Preproglucagon gene expression in pancreas and intestine diversifies at the level of post-translational processing. *J Biol Chem* 1986; **261**: 11880–9.
- 34 Baggio LL, Drucker DJ. Biology of incretins: GLP-1 and GIP. *Gastroenterology* 2007; **132**: 2131–57.
- 35 Rouille Y, Martin S, Steiner DF. Differential processing of proglucagon by the subtilisin-like prohormone convertases PC2 and PC3 to generate either glucagon or glucagon-like peptide. *J Biol Chem* 1995; **270**: 26488–96.
- 36 Campbell JE, Drucker DJ. Pharmacology, physiology, and mechanisms of incretin hormone action. *Cell Metab* 2013; **17**: 819–37.
- 37 Thorens B. Expression cloning of the pancreatic beta cell receptor for the gluco-incretin hormone glucagon-like peptide 1. *Proc Natl Acad Sci USA* 1992; **89**: 8641–5.
- 38 Richards P, Parker HE, Adriaenssens AE *et al.* Identification and characterization of GLP-1 receptor-expressing cells using a new transgenic mouse model. *Diabetes* 2014; **63**: 1224–33.
- 39 Cork SC, Richards JE, Holt MK, Gribble FM, Reimann F, Trapp S. Distribution and characterisation of Glucagon-like peptide-1 receptor expressing cells in the mouse brain. *Mol Metab* 2015; **4**: 718–31.
- 40 Nauck MA, Homberger E, Siegel EG *et al.* Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. *J Clin Endocrinol Metab* 1986; **63**: 492–8.
- 41 Vilsboll T, Krarup T, Madsbad S, Holst JJ. Both GLP-1 and GIP are insulinotropic at basal and postprandial glucose levels and contribute nearly equally to the incretin effect of a meal in healthy subjects. *Regul Pept* 2003; **114**: 115–21.
- 42 Drucker DJ. The biology of incretin hormones. *Cell Metab* 2006; **3**: 153–65.
- 43 Drucker DJ, Philippe J, Mojsos S, Chick WL, Habener JF. Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. *Proc Natl Acad Sci USA* 1987; **84**: 3434–8.
- 44 Edvell A, Lindstrom P. Initiation of increased pancreatic islet growth in young normoglycemic mice (Umea +/-). *Endocrinology* 1999; **140**: 778–83.
- 45 Perfetti R, Zhou J, Doyle ME, Egan JM. Glucagon-like peptide-1 induces cell proliferation and pancreatic-duodenum homeobox-1 expression and increases endocrine cell mass in the pancreas of old, glucose-intolerant rats. *Endocrinology* 2000; **141**: 4600–5.

- 46 Farilla L, Hui H, Bertolotto C *et al.* Glucagon-like peptide-1 promotes islet cell growth and inhibits apoptosis in Zucker diabetic rats. *Endocrinology* 2002; **143**: 4397–408.
- 47 Buteau J, Foisy S, Joly E, Prentki M. Glucagon-like peptide 1 induces pancreatic beta-cell proliferation via transactivation of the epidermal growth factor receptor. *Diabetes* 2003; **52**: 124–32.
- 48 Komatsu R, Matsuyama T, Namba M *et al.* Glucagonostatic and insulinotropic action of glucagonlike peptide I-(7-36)-amide. *Diabetes* 1989; **38**: 902–5.
- 49 Schirra J, Nicolaus M, Roggel R *et al.* Endogenous glucagon-like peptide 1 controls endocrine pancreatic secretion and antro-pyloro-duodenal motility in humans. *Gut* 2006; **55**: 243–51.
- 50 Muraro MJ, Dharmadhikari G, Grun D *et al.* A single-cell transcriptome atlas of the human pancreas. *Cell Syst* 2016; **3**: 385–94 e3.
- 51 de Heer J, Rasmussen C, Coy DH, Holst JJ. Glucagon-like peptide-1, but not glucose-dependent insulinotropic peptide, inhibits glucagon secretion via somatostatin (receptor subtype 2) in the perfused rat pancreas. *Diabetologia* 2008; **51**: 2263–70.
- 52 Muller TD, Finan B, Clemmensen C, DiMarchi RD, Tschop MH. The new biology and pharmacology of glucagon. *Physiol Rev* 2017; **97**: 721–66.
- 53 Willms B, Werner J, Holst JJ, Orskov C, Creutzfeldt W, Nauck MA. Gastric emptying, glucose responses, and insulin secretion after a liquid test meal: effects of exogenous glucagon-like peptide-1 (GLP-1)-(7-36) amide in type 2 (noninsulin-dependent) diabetic patients. *J Clin Endocrinol Metab* 1996; **81**: 327–32.
- 54 Tong J, D'Alessio D. Give the receptor a brake: slowing gastric emptying by GLP-1. *Diabetes* 2014; **63**: 407–9.
- 55 Turton MD, O'Shea D, Gunn I *et al.* A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* 1996; **379**: 69–72.
- 56 Tang-Christensen M, Vrang N, Larsen PJ. Glucagon-like peptide containing pathways in the regulation of feeding behaviour. *Int J Obes Relat Metab Disord* 2001; **25(Suppl 5)**: S42–7.
- 57 Williams DL, Baskin DG, Schwartz MW. Leptin regulation of the anorexic response to glucagon-like peptide-1 receptor stimulation. *Diabetes* 2006; **55**: 3387–93.
- 58 Chelikani PK, Haver AC, Reidelberger RD. Intravenous infusion of glucagon-like peptide-1 potently inhibits food intake, sham feeding, and gastric emptying in rats. *Am J Physiol Regul Integr Comp Physiol* 2005; **288**: R1695–706.
- 59 Sisley S, Gutierrez-Aguilar R, Scott M, D'Alessio DA, Sandoval DA, Seeley RJ. Neuronal GLP1R mediates liraglutide's anorectic but not glucose-lowering effect. *J Clin Invest* 2014; **124**: 2456–63.
- 60 Valverde I, Morales M, Clemente F *et al.* Glucagon-like peptide 1: a potent glycogenic hormone. *FEBS Lett* 1994; **349**: 313–6.
- 61 Alcantara AI, Morales M, Delgado E *et al.* Exendin-4 agonist and exendin(9-39)amide antagonist of the GLP-1(7-36)amide effects in liver and muscle. *Arch Biochem Biophys* 1997; **341**: 1–7.
- 62 Lee YS, Shin S, Shigihara T *et al.* Glucagon-like peptide-1 gene therapy in obese diabetic mice results in long-term cure of diabetes by improving insulin sensitivity and reducing hepatic gluconeogenesis. *Diabetes* 2007; **56**: 1671–9.
- 63 Gonzalez N, Acitores A, Sancho V, Valverde I, Villanueva-Penacarrillo ML. Effect of GLP-1 on glucose transport and its cell signalling in human myocytes. *Regul Pept* 2005; **126**: 203–11.
- 64 Villanueva-Penacarrillo ML, Alcantara AI, Clemente F, Delgado E, Valverde I. Potent glycogenic effect of GLP-1(7-36) amide in rat skeletal muscle. *Diabetologia* 1994; **37**: 1163–6.
- 65 Mentlein R, Gallwitz B, Schmidt WE. Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7-36)amide, peptide histidine methionine and is responsible for their degradation in human serum. *Eur J Biochem* 1993; **214**: 829–35.
- 66 Kieffer TJ, McIntosh CH, Pederson RA. Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. *Endocrinology* 1995; **136**: 3585–96.
- 67 Deacon CF, Johnsen AH, Holst JJ. Degradation of glucagon-like peptide-1 by human plasma in vitro yields an N-terminally truncated peptide that is a major endogenous metabolite in vivo. *J Clin Endocrinol Metab* 1995; **80**: 952–7.
- 68 Plamboeck A, Holst JJ, Carr RD, Deacon CF. Neutral endopeptidase 24.11 and dipeptidyl peptidase IV are both mediators of the degradation of glucagon-like peptide 1 in the anaesthetised pig. *Diabetologia* 2005; **48**: 1882–90.
- 69 Mulvihill EE, Drucker DJ. Pharmacology, physiology, and mechanisms of action of dipeptidyl peptidase-4 inhibitors. *Endocr Rev* 2014; **35**: 992–1019.
- 70 American Diabetes A. Standards of medical care in diabetes—2006. *Diabetes Care* 2006; **29(Suppl 1)**: S4–42.
- 71 Craddy P, Palin HJ, Johnson KI. Comparative effectiveness of dipeptidylpeptidase-4 inhibitors in type 2 diabetes: a systematic review and mixed treatment comparison. *Diabetes Ther* 2014; **5**: 1–41.
- 72 Runge S, Thogersen H, Madsen K, Lau J, Rudolph R. Crystal structure of the ligand-bound glucagon-like peptide-1 receptor extracellular domain. *J Biol Chem* 2008; **283**: 11340–7.
- 73 Neidigh JW, Fesinmeyer RM, Prickett KS, Andersen NH. Exendin-4 and glucagon-like-peptide-1: NMR structural comparisons in the solution and micelle-associated states. *Biochemistry* 2001; **40**: 13188–200.
- 74 West GM, Willard FS, Sloop KW, Showalter AD, Pascal BD, Griffin PR. Glucagon-like peptide-1 receptor ligand interactions: structural cross talk between ligands and the extracellular domain. *PLoS ONE* 2014; **9**: e105683.
- 75 Al-Sabah S, Donnelly D. A model for receptor-peptide binding at the glucagon-like peptide-1 (GLP-1) receptor through the analysis of truncated ligands and receptors. *Br J Pharmacol* 2003; **140**: 339–46.
- 76 Furman BL. The development of Byetta (exenatide) from the venom of the Gila monster as an anti-diabetic agent. *Toxicol* 2012; **59**: 464–71.
- 77 Gyskiewicz KA, Coleman CI. Focus on exenatide. A novel incretin mimetic hormone for the treatment of type 2 diabetes. *Formulary* 2005; **40**: 86–90.
- 78 Jose B, Tahrani AA, Piya MK, Barnett AH. Exenatide once weekly: clinical outcomes and patient satisfaction. *Patient Prefer Adherence* 2010; **4**: 313–24.
- 79 Fineman MS, Shen LZ, Taylor K, Kim DD, Baron AD. Effectiveness of progressive dose-escalation of exenatide (exendin-4) in reducing dose-limiting side effects in subjects with type 2 diabetes. *Diabetes Metab Res Rev* 2004; **20**: 411–7.

- 80 Russell-Jones D, Cuddihy RM, Hanefeld M *et al.* Efficacy and safety of exenatide once weekly versus metformin, pioglitazone, and sitagliptin used as monotherapy in drug-naive patients with type 2 diabetes (DURATION-4): a 26-week double-blind study. *Diabetes Care* 2012; **35**: 252–8.
- 81 McCarty D, Coleman M, Boland CL. Lixisenatide: a new daily GLP-1 agonist for type 2 diabetes management. *Ann Pharmacother* 2017; **51**: 401–9.
- 82 Rosenstock J, Raccach D, Koranyi L *et al.* Efficacy and safety of lixisenatide once daily versus exenatide twice daily in type 2 diabetes inadequately controlled on metformin: a 24-week, randomized, open-label, active-controlled study (GetGoal-X). *Diabetes Care* 2013; **36**: 2945–51.
- 83 Kim D, MacConnell L, Zhuang D *et al.* Effects of once-weekly dosing of a long-acting release formulation of exenatide on glucose control and body weight in subjects with type 2 diabetes. *Diabetes Care* 2007; **30**: 1487–93.
- 84 Drucker DJ, Buse JB, Taylor K *et al.* Exenatide once weekly versus twice daily for the treatment of type 2 diabetes: a randomised, open-label, non-inferiority study. *Lancet* 2008; **372**: 1240–50.
- 85 Muller TD, Clemmensen C, Finan B, DiMarchi RD, Tschop MH. Anti-obesity therapy: from rainbow pills to polyagonists. *Pharmacol Rev* 2018; **70**: 712–46.
- 86 Nauck M, Frid A, Hermansen K *et al.* Efficacy and safety comparison of liraglutide, glimepiride, and placebo, all in combination with metformin, in type 2 diabetes: the LEAD (liraglutide effect and action in diabetes)-2 study. *Diabetes Care* 2009; **32**: 84–90.
- 87 Buse JB, Rosenstock J, Sesti G *et al.* Liraglutide once a day versus exenatide twice a day for type 2 diabetes: a 26-week randomised, parallel-group, multinational, open-label trial (LEAD-6). *Lancet* 2009; **374**: 39–47.
- 88 Buse JB, Nauck M, Forst T *et al.* Exenatide once weekly versus liraglutide once daily in patients with type 2 diabetes (DURATION-6): a randomised, open-label study. *Lancet* 2013; **381**: 117–24.
- 89 McGill JB. Insights from the Liraglutide Clinical Development Program—the Liraglutide Effect and Action in Diabetes (LEAD) studies. *Postgrad Med* 2009; **121**: 16–25.
- 90 Bush MA, Matthews JE, De Boever EH *et al.* Safety, tolerability, pharmacodynamics and pharmacokinetics of albiglutide, a long-acting glucagon-like peptide-1 mimetic, in healthy subjects. *Diabetes Obes Metab* 2009; **11**: 498–505.
- 91 Pratley RE, Nauck MA, Barnett AH *et al.* Once-weekly albiglutide versus once-daily liraglutide in patients with type 2 diabetes inadequately controlled on oral drugs (HARMONY 7): a randomised, open-label, multicentre, non-inferiority phase 3 study. *Lancet Diabetes Endocrinol* 2014; **2**: 289–97.
- 92 Dungan KM, Povedano ST, Forst T *et al.* Once-weekly dulaglutide versus once-daily liraglutide in metformin-treated patients with type 2 diabetes (AWARD-6): a randomised, open-label, phase 3, non-inferiority trial. *Lancet* 2014; **384**: 1349–57.
- 93 Lau J, Bloch P, Schaffer L *et al.* Discovery of the once-weekly Glucagon-Like Peptide-1 (GLP-1) analogue semaglutide. *J Med Chem* 2015; **58**: 7370–80.
- 94 Sorli C, Harashima SI, Tsoukas GM *et al.* Efficacy and safety of once-weekly semaglutide monotherapy versus placebo in patients with type 2 diabetes (SUSTAIN 1): a double-blind, randomised, placebo-controlled, parallel-group, multinational, multicentre phase 3a trial. *Lancet Diabetes Endocrinol* 2017; **5**: 251–60.
- 95 Pratley RE, Aroda VR, Lingvay I *et al.* Semaglutide versus dulaglutide once weekly in patients with type 2 diabetes (SUSTAIN 7): a randomised, open-label, phase 3b trial. *Lancet Diabetes Endocrinol* 2018; **6**: 275–86.
- 96 Trujillo JM, Nuffer W, Ellis SL. GLP-1 receptor agonists: a review of head-to-head clinical studies. *Ther Adv Endocrinol Metab* 2015; **6**: 19–28.
- 97 Talsania T, Anini Y, Siu S, Drucker DJ, Brubaker PL. Peripheral exendin-4 and peptide YY(3-36) synergistically reduce food intake through different mechanisms in mice. *Endocrinology* 2005; **146**: 3748–56.
- 98 Neary NM, Small CJ, Druce MR *et al.* Peptide YY3-36 and glucagon-like peptide-17-36 inhibit food intake additively. *Endocrinology* 2005; **146**: 5120–7.
- 99 Steinert RE, Poller B, Castelli MC, Drewe J, Beglinger C. Oral administration of glucagon-like peptide 1 or peptide YY 3-36 affects food intake in healthy male subjects. *Am J Clin Nutr* 2010; **92**: 810–7.
- 100 De Silva A, Salem V, Long CJ *et al.* The gut hormones PYY 3-36 and GLP-1 7-36 amide reduce food intake and modulate brain activity in appetite centers in humans. *Cell Metab* 2011; **14**: 700–6.
- 101 Reidelberger RD, Haver AC, Apenteng BA, Anders KL, Stenson SM. Effects of exendin-4 alone and with peptide YY(3-36) on food intake and body weight in diet-induced obese rats. *Obesity (Silver Spring)* 2011; **19**: 121–7.
- 102 Bello NT, Kemm MH, Ofeldt EM, Moran TH. Dose combinations of exendin-4 and salmon calcitonin produce additive and synergistic reductions in food intake in nonhuman primates. *Am J Physiol Regul Integr Comp Physiol* 2010; **299**: R945–52.
- 103 Muller TD, Sullivan LM, Habegger K *et al.* Restoration of leptin responsiveness in diet-induced obese mice using an optimized leptin analog in combination with exendin-4 or FGF21. *J Pept Sci* 2012; **18**: 383–93.
- 104 Clemmensen C, Finan B, Fischer K *et al.* Dual melanocortin-4 receptor and GLP-1 receptor agonism amplifies metabolic benefits in diet-induced obese mice. *EMBO Mol Med* 2015; **7**: 288–98.
- 105 Capozzi ME, DiMarchi RD, Tschop MH, Finan B, Campbell JE. Targeting the incretin/glucagon system with triagonists to treat diabetes. *Endocr Rev* 2018; [Epub ahead of print]. <http://doi.org/10.1210/er.2018-00117>.
- 106 Kimball C, Murlin J. Aqueous extracts of pancreas III. Some precipitation reactions of insulin. *J Biol Chem* 1923; **58**: 337–48.
- 107 Tuomi T, Miettinen PJ, Hakaste L, Groop L. Atypical forms of diabetes. In: De Groot LJ, Chrousos G, Dungan K *et al.*, eds. *Endotext*. South Dartmouth (MA):MDText.com, Inc, 2000.
- 108 Unger RH, Aguilar-Parada E, Muller WA, Eisentraut AM. Studies of pancreatic alpha cell function in normal and diabetic subjects. *J Clin Invest* 1970; **49**: 837–48.
- 109 Gerich JE, Langlois M, Noacco C, Lorenzi M, Karam JH, Korsham PH. Comparison of the suppressive effects of elevated plasma glucose and free fatty acid levels on glucagon secretion in normal and insulin-dependent diabetic subjects. Evidence for selective alpha-cell insensitivity to glucose in diabetes mellitus. *J Clin Invest* 1976; **58**: 320–5.
- 110 Mu J, Jiang G, Brady E *et al.* Chronic treatment with a glucagon receptor antagonist lowers glucose and moderately

- raises circulating glucagon and glucagon-like peptide 1 without severe alpha cell hypertrophy in diet-induced obese mice. *Diabetologia* 2011; **54**: 2381–91.
- 111 Okamoto H, Kim J, Aglione J *et al.* Glucagon receptor blockade with a human antibody normalizes blood glucose in diabetic mice and monkeys. *Endocrinology* 2015; **156**: 2781–94.
- 112 Rivera N, Everett-Grueter CA, Edgerton DS *et al.* A novel glucagon receptor antagonist, NNC 25-0926, blunts hepatic glucose production in the conscious dog. *J Pharmacol Exp Ther* 2007; **321**: 743–52.
- 113 Kelly RP, Garhyan P, Raddad E *et al.* Short-term administration of the glucagon receptor antagonist LY2409021 lowers blood glucose in healthy people and in those with type 2 diabetes. *Diabetes Obes Metab* 2015; **17**: 414–22.
- 114 Kostic A, King TA, Yang F *et al.* A first-in-human pharmacodynamic and pharmacokinetic study of a fully human anti-glucagon receptor monoclonal antibody in normal healthy volunteers. *Diabetes Obes Metab* 2018; **20**: 283–91.
- 115 Amatuzio DS, Grande F, Wada S. Effect of glucagon on the serum lipids in essential hyperlipemia and in hypercholesterolemia. *Metabolism* 1962; **11**: 1240–9.
- 116 Caren R, Corbo L. Glucagon and cholesterol metabolism. *Metabolism* 1960; **9**: 938–45.
- 117 Guettet C, Rostaqui N, Navarro N, Lecuyer B, Mathe D. Effect of chronic glucagon administration on the metabolism of triacylglycerol-rich lipoproteins in rats fed a high sucrose diet. *J Nutr* 1991; **121**: 24–30.
- 118 Habegger KM, Stemmer K, Cheng C *et al.* Fibroblast growth factor 21 mediates specific glucagon actions. *Diabetes* 2013; **62**: 1453–63.
- 119 Hagen JH. Effect of glucagon on the metabolism of adipose tissue. *J Biol Chem* 1961; **236**: 1023–7.
- 120 Richter WO, Robl H, Schwandt P. Human glucagon and vasoactive intestinal polypeptide (VIP) stimulate free fatty acid release from human adipose tissue in vitro. *Peptides* 1989; **10**: 333–5.
- 121 Pegorier JP, Garcia-Garcia MV, Prip-Buus C, Duee PH, Kohl C, Girard J. Induction of ketogenesis and fatty acid oxidation by glucagon and cyclic AMP in cultured hepatocytes from rabbit fetuses. Evidence for a decreased sensitivity of carnitine palmitoyltransferase I to malonyl-CoA inhibition after glucagon or cyclic AMP treatment. *Biochem J* 1989; **264**: 93–100.
- 122 Stunkard AJ, Van Itallie TB, Reis BB. The mechanism of satiety: effect of glucagon on gastric hunger contractions in man. *Proc Soc Exp Biol Med* 1955; **89**: 258–61.
- 123 Schulman JL, Carleton JL, Whitney G, Whitehorn JC. Effect of glucagon on food intake and body weight in man. *J Appl Physiol* 1957; **11**: 419–21.
- 124 Penick SB, Hinkle LE Jr. Depression of food intake induced in healthy subjects by glucagon. *N Engl J Med* 1961; **264**: 893–7.
- 125 Nair KS. Hyperglucagonemia increases resting metabolic rate in man during insulin deficiency. *J Clin Endocrinol Metab* 1987; **64**: 896–901.
- 126 Martin JR, Novin D. Decreased feeding in rats following hepatic-portal infusion of glucagon. *Physiol Behav* 1977; **19**: 461–6.
- 127 Davidson IW, Salter JM, Best CH. Calorigenic action of glucagon. *Nature* 1957; **180**: 1124.
- 128 Kuroshima A, Yahata T. Thermogenic responses of brown adipocytes to noradrenaline and glucagon in heat-acclimated and cold-acclimated rats. *Jpn J Physiol* 1979; **29**: 683–90.
- 129 Billington CJ, Briggs JE, Link JG, Levine AS. Glucagon in physiological concentrations stimulates brown fat thermogenesis in vivo. *Am J Physiol* 1991; **261**: R501–7.
- 130 Dicker A, Zhao J, Cannon B, Nedergaard J. Apparent thermogenic effect of injected glucagon is not due to a direct effect on brown fat cells. *Am J Physiol* 1998; **275**: R1674–82.
- 131 Salem V, Izzi-Engbeaya C, Coello C *et al.* Glucagon increases energy expenditure independently of brown adipose tissue activation in humans. *Diabetes Obes Metab* 2016; **18**: 72–81.
- 132 Jelinek LJ, Lok S, Rosenberg GB *et al.* Expression cloning and signaling properties of the rat glucagon receptor. *Science* 1993; **259**: 1614–6.
- 133 Hjorth SA, Adelhorst K, Pedersen BB, Kirk O, Schwartz TW. Glucagon and glucagon-like peptide 1: selective receptor recognition via distinct peptide epitopes. *J Biol Chem* 1994; **269**: 30121–4.
- 134 Day JW, Ottaway N, Patterson JT *et al.* A new glucagon and GLP-1 co-agonist eliminates obesity in rodents. *Nat Chem Biol* 2009; **5**: 749–57.
- 135 Clemmensen C, Chabenne J, Finan B *et al.* GLP-1/glucagon coagonism restores leptin responsiveness in obese mice chronically maintained on an obesogenic diet. *Diabetes* 2014; **63**: 1422–7.
- 136 Gault VA, Bhat VK, Irwin N, Flatt PR. A novel glucagon-like peptide-1 (GLP-1)/glucagon hybrid peptide with triple-acting agonist activity at glucose-dependent insulinotropic polypeptide, GLP-1, and glucagon receptors and therapeutic potential in high fat-fed mice. *J Biol Chem* 2013; **288**: 35581–91.
- 137 Henderson SJ, Konkar A, Hornigold DC *et al.* Robust anti-obesity and metabolic effects of a dual GLP-1/glucagon receptor peptide agonist in rodents and non-human primates. *Diabetes Obes Metab* 2016; **18**: 1176–90.
- 138 Ambery PD, Klamm S, Posch MG *et al.* MEDI0382, a GLP-1-glucagon receptor dual agonist, meets safety and tolerability endpoints in a single-dose, healthy-subject, randomized, phase 1 study. *Br J Clin Pharmacol* 2018; **84**: 2325–35.
- 139 Pocai A, Carrington PE, Adams JR *et al.* Glucagon-like peptide 1/glucagon receptor dual agonism reverses obesity in mice. *Diabetes* 2009; **58**: 2258–66.
- 140 Baldissera FG, Holst JJ, Knuhtsen S, Hilsted L, Nielsen OV. Oxyntomodulin (glicentin-(33-69)): pharmacokinetics, binding to liver cell membranes, effects on isolated perfused pig pancreas, and secretion from isolated perfused lower small intestine of pigs. *Regul Pept* 1988; **21**: 151–66.
- 141 Gros L, Thorens B, Bataille D, Kervran A. Glucagon-like peptide-1-(7-36) amide, oxyntomodulin, and glucagon interact with a common receptor in a somatostatin-secreting cell line. *Endocrinology* 1993; **133**: 631–8.
- 142 Baggio LL, Huang Q, Brown TJ, Drucker DJ. Oxyntomodulin and glucagon-like peptide-1 differentially regulate murine food intake and energy expenditure. *Gastroenterology* 2004; **127**: 546–58.
- 143 Schepp W, Dehne K, Riedel T, Schmidler J, Schaffer K, Classen M. Oxyntomodulin: a cAMP-dependent stimulus of

- rat parietal cell function via the receptor for glucagon-like peptide-1 (7-36)NH₂. *Digestion* 1996; **57**: 398–405.
- 144 Brandt SJ, Gotz A, Tschop MH, Muller TD. Gut hormone polyagonists for the treatment of type 2 diabetes. *Peptides* 2018; **100**: 190–201.
- 145 Hay DL, Chen S, Lutz TA, Parkes DG, Roth JD. Amylin: pharmacology, physiology, and clinical potential. *Pharmacol Rev* 2015; **67**: 564–600.
- 146 Rushing PA, Seeley RJ, Air EL, Lutz TA, Woods SC. Acute 3rd-ventricular amylin infusion potently reduces food intake but does not produce aversive consequences. *Peptides* 2002; **23**: 985–8.
- 147 Mack C, Wilson J, Athanacio J *et al.* Pharmacological actions of the peptide hormone amylin in the long-term regulation of food intake, food preference, and body weight. *Am J Physiol Regul Integr Comp Physiol* 2007; **293**: R1855–63.
- 148 Lutz TA, Mollet A, Rushing PA, Riediger T, Scharrer E. The anorectic effect of a chronic peripheral infusion of amylin is abolished in area postrema/nucleus of the solitary tract (AP/NTS) lesioned rats. *Int J Obes Relat Metab Disord* 2001; **25**: 1005–11.
- 149 Mack CM, Soares CJ, Wilson JK *et al.* Divalintide (AC2307), a novel amylin-mimetic peptide: enhanced pharmacological properties over native amylin to reduce food intake and body weight. *Int J Obes (Lond)* 2010; **34**: 385–95.
- 150 Trevasakis JL, Mack CM, Sun C *et al.* Improved glucose control and reduced body weight in rodents with dual mechanism of action peptide hybrids. *PLoS ONE* 2013; **8**: e78154.
- 151 Sun C, Trevasakis JL, Jodka CM *et al.* Bifunctional PEGylated exenatide-amylinomimetic hybrids to treat metabolic disorders: an example of long-acting dual hormonal therapeutics. *J Med Chem* 2013; **56**: 9328–41.
- 152 Little TJ, Horowitz M, Feinle-Bisset C. Role of cholecystokinin in appetite control and body weight regulation. *Obes Rev* 2005; **6**: 297–306.
- 153 Varro A, Ardill JE. Gastrin: an analytical review. *Ann Clin Biochem* 2003; **40**: 472–80.
- 154 Irwin N, Hunter K, Montgomery IA, Flatt PR. Comparison of independent and combined metabolic effects of chronic treatment with (pGlu-Gln)-CCK-8 and long-acting GLP-1 and GIP mimetics in high fat-fed mice. *Diabetes Obes Metab* 2013; **15**: 650–9.
- 155 Trevasakis JL, Sun C, Athanacio J *et al.* Synergistic metabolic benefits of an exenatide analogue and cholecystokinin in diet-induced obese and leptin-deficient rodents. *Diabetes Obes Metab* 2015; **17**: 61–73.
- 156 Irwin N, Pathak V, Flatt PR. A novel CCK-8/GLP-1 hybrid peptide exhibiting prominent insulinotropic, glucose-lowering, and satiety actions with significant therapeutic potential in high-fat-fed mice. *Diabetes* 2015; **64**: 2996–3009.
- 157 Hornigold DC, Roth E, Howard V *et al.* A GLP-1:CCK fusion peptide harnesses the synergistic effects on metabolism of CCK-1 and GLP-1 receptor agonism in mice. *Appetite* 2018; **127**: 334–40.
- 158 Dalboge LS, Almholt DL, Neerup TS, Vrang N, Jelsing J, Fosgerau K. The novel GLP-1-gastrin dual agonist ZP3022 improves glucose homeostasis and increases beta-cell mass without affecting islet number in db/db mice. *J Pharmacol Exp Ther* 2014; **350**: 353–60.
- 159 Skarbaliene J, Secher T, Jelsing J *et al.* The anti-diabetic effects of GLP-1-gastrin dual agonist ZP3022 in ZDF rats. *Peptides* 2015; **69**: 47–55.
- 160 Dupre J, Ross SA, Watson D, Brown JC. Stimulation of insulin secretion by gastric inhibitory polypeptide in man. *J Clin Endocrinol Metab* 1973; **37**: 826–8.
- 161 Lardinois CK, Starich GH, Mazzaferri EL. The postprandial response of gastric inhibitory polypeptide to various dietary fats in man. *J Am Coll Nutr* 1988; **7**: 241–7.
- 162 Meier JJ, Gallwitz B, Siepmann N *et al.* Gastric inhibitory polypeptide (GIP) dose-dependently stimulates glucagon secretion in healthy human subjects at euglycaemia. *Diabetologia* 2003; **46**: 798–801.
- 163 Christensen M, Vedtofte L, Holst JJ, Vilsboll T, Knop FK. Glucose-dependent insulinotropic polypeptide: a bifunctional glucose-dependent regulator of glucagon and insulin secretion in humans. *Diabetes* 2011; **60**: 3103–9.
- 164 Christensen MB, Calanna S, Holst JJ, Vilsboll T, Knop FK. Glucose-dependent insulinotropic polypeptide: blood glucose stabilizing effects in patients with type 2 diabetes. *J Clin Endocrinol Metab* 2014; **99**: E418–26.
- 165 Eckel RH, Fujimoto WY, Brunzell JD. Gastric inhibitory polypeptide enhanced lipoprotein lipase activity in cultured preadipocytes. *Diabetes* 1979; **28**: 1141–2.
- 166 Bailey CJ, Flatt PR, Kwasowski P, Powell CJ, Marks V. Immunoreactive gastric inhibitory polypeptide and K cell hyperplasia in obese hyperglycaemic (ob/ob) mice fed high fat and high carbohydrate cafeteria diets. *Acta Endocrinol (Copenh)* 1986; **112**: 224–9.
- 167 Flatt PR, Bailey CJ, Kwasowski P, Swanston-Flatt SK, Marks V. Abnormalities of GIP in spontaneous syndromes of obesity and diabetes in mice. *Diabetes* 1983; **32**: 433–5.
- 168 Miyawaki K, Yamada Y, Ban N *et al.* Inhibition of gastric inhibitory polypeptide signaling prevents obesity. *Nat Med* 2002; **8**: 738–42.
- 169 Calanna S, Christensen M, Holst JJ *et al.* Secretion of glucose-dependent insulinotropic polypeptide in patients with type 2 diabetes: systematic review and meta-analysis of clinical studies. *Diabetes Care* 2013; **36**: 3346–52.
- 170 Kim SJ, Nian C, Karunakaran S, Clee SM, Isales CM, McIntosh CH. GIP-overexpressing mice demonstrate reduced diet-induced obesity and steatosis, and improved glucose homeostasis. *PLoS ONE* 2012; **7**: e40156.
- 171 Martin CM, Irwin N, Flatt PR, Gault VA. A novel acylated form of (d-Ala(2))GIP with improved antidiabetic potential, lacking effect on body fat stores. *Biochim Biophys Acta* 2013; **1830**: 3407–13.
- 172 Renner S, Fehlings C, Herbach N *et al.* Glucose intolerance and reduced proliferation of pancreatic beta-cells in transgenic pigs with impaired glucose-dependent insulinotropic polypeptide function. *Diabetes* 2010; **59**: 1228–38.
- 173 Ambati S, Duan J, Hartzell DL, Choi YH, Della-Fera MA, Baile CA. GIP-dependent expression of hypothalamic genes. *Physiol Res* 2011; **60**: 941–50.
- 174 Gault VA, Kerr BD, Harriott P, Flatt PR. Administration of an acylated GLP-1 and GIP preparation provides added beneficial glucose-lowering and insulinotropic actions over single incretins in mice with Type 2 diabetes and obesity. *Clin Sci (Lond)* 2011; **121**: 107–17.
- 175 Nauck MA, Bartels E, Orskov C, Ebert R, Creutzfeldt W. Additive insulinotropic effects of exogenous synthetic human gastric inhibitory polypeptide and glucagon-like

- peptide-1-(7-36) amide infused at near-physiological insulinotropic hormone and glucose concentrations. *J Clin Endocrinol Metab* 1993; **76**: 912–7.
- 176 Mentis N, Vardarli I, Kothe LD *et al.* GIP does not potentiate the antidiabetic effects of GLP-1 in hyperglycemic patients with type 2 diabetes. *Diabetes* 2011; **60**: 1270–6.
- 177 Finan B, Ma T, Ottaway N *et al.* Unimolecular dual incretins maximize metabolic benefits in rodents, monkeys, and humans. *Sci Transl Med* 2013; **5**: 209ra151.
- 178 Frias JP, Bastyr EJ 3rd, Vignati L *et al.* The sustained effects of a dual GIP/GLP-1 receptor agonist, NNC0090-2746, in patients with type 2 diabetes. *Cell Metab* 2017; **26**: 343–52 e2.
- 179 Bhat VK, Kerr BD, Flatt PR, Gault VA. A novel GIP-oxintomodulin hybrid peptide acting through GIP, glucagon and GLP-1 receptors exhibits weight reducing and anti-diabetic properties. *Biochem Pharmacol* 2013; **85**: 1655–62.
- 180 Bhat VK, Kerr BD, Vasu S, Flatt PR, Gault VA. A DPP-IV-resistant triple-acting agonist of GIP, GLP-1 and glucagon receptors with potent glucose-lowering and insulinotropic actions in high-fat-fed mice. *Diabetologia* 2013; **56**: 1417–24.
- 181 Finan B, Yang B, Ottaway N *et al.* A rationally designed monomeric peptide triagonist corrects obesity and diabetes in rodents. *Nat Med* 2015; **21**: 27–36.
- 182 Jall S, Sachs S, Clemmensen C *et al.* Monomeric GLP-1/GIP/glucagon triagonism corrects obesity, hepatosteatosis, and dyslipidemia in female mice. *Mol Metab* 2017; **6**: 440–6.
- 183 Choi Y, Lee JS, Kim JK *et al.* Potent body weight loss and efficacy in a NASH animal model by a novel long-acting GLP-1/Glucagon/GIP triple-agonist (HM15211). *American Diabetes Association's (ADA) 77th Scientific Sessions*. San Diego. 2017.
- 184 Kim EA, Lee S, Lee SH *et al.* Neuroprotective Effects of HM15211, a Novel Long-Acting GLP-1/GIP/Glucagon Triple Agonist in the Neurodegenerative Disease Models. *American Diabetes Association's 77th Scientific Session*. San Diego. 2017.
- 185 Mauvais-Jarvis F. Estrogen and androgen receptors: regulators of fuel homeostasis and emerging targets for diabetes and obesity. *Trends Endocrinol Metab* 2011; **22**: 24–33.
- 186 Tiano JP, Delghingaro-Augusto V, Le May C *et al.* Estrogen receptor activation reduces lipid synthesis in pancreatic islets and prevents beta cell failure in rodent models of type 2 diabetes. *J Clin Invest* 2011; **121**: 3331–42.
- 187 Schacke H, Docke WD, Asadullah K. Mechanisms involved in the side effects of glucocorticoids. *Pharmacol Ther* 2002; **96**: 23–43.
- 188 Mullur R, Liu YY, Brent GA. Thyroid hormone regulation of metabolism. *Physiol Rev* 2014; **94**: 355–82.
- 189 Finan B, Yang B, Ottaway N *et al.* Targeted estrogen delivery reverses the metabolic syndrome. *Nat Med* 2012; **18**: 1847–56.
- 190 Quarta C, Clemmensen C, Zhu Z *et al.* Molecular integration of incretin and glucocorticoid action reverses immunometabolic dysfunction and obesity. *Cell Metab* 2017; **26**: 620–32 e6.
- 191 Finan B, Clemmensen C, Zhu Z *et al.* Chemical hybridization of glucagon and thyroid hormone optimizes therapeutic impact for metabolic disease. *Cell* 2016; **167**: 843–57 e14.
- 192 Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. *Annu Rev Immunol* 2011; **29**: 415–45.
- 193 Thaler JP, Yi CX, Schur EA *et al.* Obesity is associated with hypothalamic injury in rodents and humans. *J Clin Invest* 2012; **122**: 153–62.
- 194 Targher G, Bertolini L, Padovani R *et al.* Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. *Diabetes Care* 2007; **30**: 1212–8.

Correspondence: Kerstin Stemmer and Matthias H. Tschöp, Institute for Diabetes and Obesity, Helmholtz Zentrum München, Ingolstädter Landstraße 1, Neuherberg 85764, Germany. (fax: +49-89-3187-2182; e-mail: kerstin.stemmer@helmholtz-muenchen.de and matthias.tschoep@helmholtz-muenchen.de) ■