

## REVIEW

# How glucagon-like peptide 1 receptor agonists work

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## Abstract

In recent years, glucagon-like peptide 1 receptor agonists (GLP-1RAs) have become central in the treatment of type 2 diabetes (T2D). In addition to their glucose-lowering properties with low risk of hypoglycaemia, GLP-1RAs reduce body weight and show promising results in reducing cardiovascular risk and renal complications in high-risk individuals with T2D. These findings have changed guidelines on T2D management over the last years, and GLP-1RAs are now widely used in overweight patients with T2D as well as in patients with T2D and cardiovascular disease regardless of glycaemic control. The currently available GLP-1RAs have different pharmacokinetic profiles and differ in their ability to improve glycaemia, reduce body weight and in their cardio- and renal protective potentials. Understanding how these agents work, including insights into their pleiotropic effects on T2D pathophysiology, may improve their clinical utilisation and be useful for exploring other indications such as non-alcoholic steatohepatitis and neurodegenerative disorders. In this review, we provide an overview of approved GLP-1RAs, their clinical effects and mode of action, and we offer insights into the potential of GLP-1RAs for other indications than T2D. Finally, we will discuss the emerging data and therapeutic potential of using GLP-1RAs in combinations with other receptor agonists.

## Key Words

- ▶ diabetes
- ▶ cardiovascular
- ▶ metabolism
- ▶ inflammation
- ▶ obesity

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## Introduction

Glucagon-like peptide 1 (GLP-1) is a gut-derived glucoregulatory hormone secreted in response to food consumption. Human GLP-1 is synthesised from the proglucagon gene and is secreted from the enteroendocrine L cells (1). Some preproglucagon-expressing neurons in the nucleus tractus solitarius of the brain stem have also been shown to synthesise GLP-1 (2). Due to rapid breakdown by dipeptidyl peptidase 4 (DPP-4) and renal clearance, the half-life of circulating GLP-1 is only 1–2 min. Thus, only 10% of the native GLP-1 released from the enteroendocrine L cells reaches the systemic circulation (1). Activation of the GLP-1 receptor (GLP-1R) on the beta-cell enhances glucose-dependent secretion of

insulin, thereby improving beta-cell sensitivity to glucose (1). In addition, GLP-1 exhibits a glucagonostatic effect through a glucose-dependent inhibition of glucagon release (3) which, compared to GLP-1-induced insulin secretion, may be equally important for the overall plasma glucose-lowering effect of GLP-1 (4). In normal physiology, GLP-1 modifies gastrointestinal function by decreasing motility of the stomach and intestine, thereby delaying gastric emptying and reducing postprandial plasma glucose excursions (5). Brain-derived GLP-1 acts as a neurotransmitter and can target multiple GLP-1Rs throughout the central nervous system (CNS), including effects on satiety (6). In addition, GLP-1R activation in

the brain has been linked to neuroprotective properties in experimental neurodegenerative disease models (6). Deduced from both preclinical and human studies, GLP-1 has extra-pancreatic effects in the gut, kidney, nervous system, heart and immune system (Fig. 1) (2). Multiple GLP-1R agonists (GLP-1RAs) utilising the plasma glucose-lowering and body weight-lowering effects of GLP-1 have been developed, and today, we have more than 15 years of clinical experience with GLP-1RAs for the treatment of T2D and 6 years of clinical experience with GLP-1RA-based treatment of obesity. Despite the long clinical experience with GLP-1RAs, the exact mechanisms by which GLP-1RAs exert their effects are still debated (6).

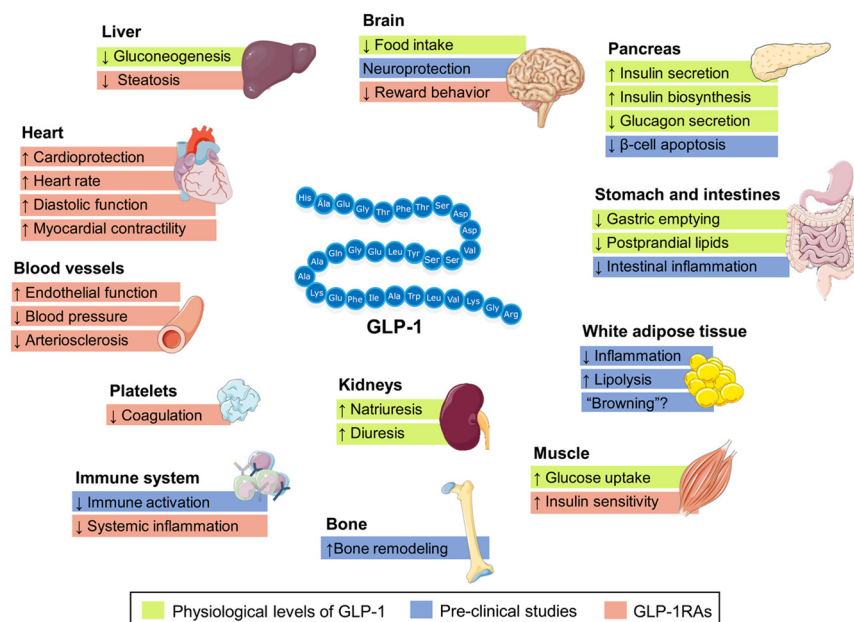
whereas long-acting GLP-1RAs lead to continuous receptor activation over a 24-h period. Liraglutide, semaglutide and dulaglutide are – except for few modifications – highly similar to the structure of human GLP-1, while exenatide and lixisenatide are based on exendin-4, a naturally occurring peptide derived from the saliva of the Gila monster, and only share around 50% homology with the human GLP-1 molecule. Consequently, the exendin-based compounds cause a higher degree of antibody formation, but it is questionable whether this is important for efficacy and safety (7, 8). The pharmacokinetic properties of approved GLP-1RAs are listed in Table 1.

### Currently available GLP-1RAs

The first GLP-1RA for the treatment of T2D, exenatide twice daily, was approved by the United States Food and Drug Administration (FDA) in 2005. Since then, several GLP-1RAs have been developed and approved for the treatment of T2D, while albiglutide has been withdrawn due to commercial reasons. Until recently, all available GLP-1RAs were subcutaneously administered; however, the GLP-1RA semaglutide (see below) is now available as an oral formulation. GLP-1RAs can be categorised based on molecular size, chemical structure and duration of action. As outlined below, the pharmacokinetic properties are important for the mode of action and the efficacy of the individual GLP-1RA. Short-acting GLP-1RAs are characterised by intermittent activation of the GLP-1R,

### Short-acting GLP-1RAs

The short-acting exenatide is identical to the exendin-4 structure, while lixisenatide has been modified from exendin-4 (deletion of proline and an addition of six lysine amino acids at the carboxyl terminus) to make them resistant to degradation by DPP-4 without compromising their GLP-1R activating potencies (9). They are administered once (lixisenatide) or twice daily (exenatide) in relation to meals and have plasma half-lives in the range of 2.4–3 h resulting in low plasma drug concentrations between injections. Because of their intermittent stimulation of the GLP-1R, short-acting GLP-1RAs retain their ability to delay gastric emptying leading to lower postprandial glucose excursions compared with the long-acting GLP-1RAs, which are associated with the development of tolerance (tachyphylaxis) to GLP-1's effect on gastric emptying (10).



**Figure 1** Possible actions of glucagon-like peptide 1 (GLP-1) and the GLP-1 receptor agonists (GLP-1RAs) on various tissue. The applied colour code indicates whether the effect on the target tissue has been observed in preclinical studies (blue boxes), at physiological levels of GLP-1 in clinical studies (green boxes) or after treatment with GLP-1RAs (red boxes) (1, 2). The figure illustrates GLP-1 (7–36) amide.

**Table 1** Pharmacokinetic properties of approved GLP-1RAs.

GLP-1RAs	Approval year		Molecular weight (Da)	Pharmacokinetic components	Administration schedule	Dose	Pharmacokinetics (single-dose administration)			Antibody development (% of patients)
	FDA	EMA					Time to peak	Half-life	Elimination	
<b>Short-acting GLP-1RAs</b>										
Exenatide twice daily	2005	2006	4187	Peptide from the Gila lizard (53% homology with native GLP-1)	Twice daily	5–10 µg	2.1–2.2 h	2.4 h	Mainly renal	35
<b>Long-acting GLP-1RAs</b>										
Lixisenatide	2016	2013	4859	Exenatide plus poly-lysine tail	Once daily	10–20 µg	≈ 2 h	3 h	Mainly renal	56–70
Liraglutide	2010	2009	3751	Modified GLP-1 with free fatty acid side chain attached (97% homology with native GLP-1)	Once daily	0.6–3 mg	11.0–13.8 h	13 h	Peptide hydrolysis, renal (6%)+faecal (5%)	8.6
Exenatide once weekly	2012	2011	4187	See exenatide twice daily	Once weekly	2 mg	Slow*	2.4 h	Mainly renal	57
Dulaglutide	2014	2014	59,671	Modified GLP-1 with attached immunoglobulin (Fc) fragment (~90% homology with native GLP-1)	Once weekly	0.75–1.5 mg	3–5 days	90 h	Peptidases and renal	1.6
Semaglutide once weekly	2017	2019	4113	Modified GLP-1 with free fatty acid side chain attached (94% homology to native GLP-1)	Once weekly	0.5–1 mg	24 h	165 h	Peptidases and renal	0.01–3.50
Oral semaglutide	2019	2020	4114	See semaglutide once weekly	Once daily	3–14 mg	1–4 h	165–185 h	Peptidases	0.5

Has been withdrawn in July 2018 for commercial reasons. \*\*Not formally assessed. EMA, European Medicines Agency; FDA, U.S. Food and Drug Administration; GLP-1, glucagon-like peptide-1; GLP-1RAs, glucagon-like peptide-1 receptor agonists.

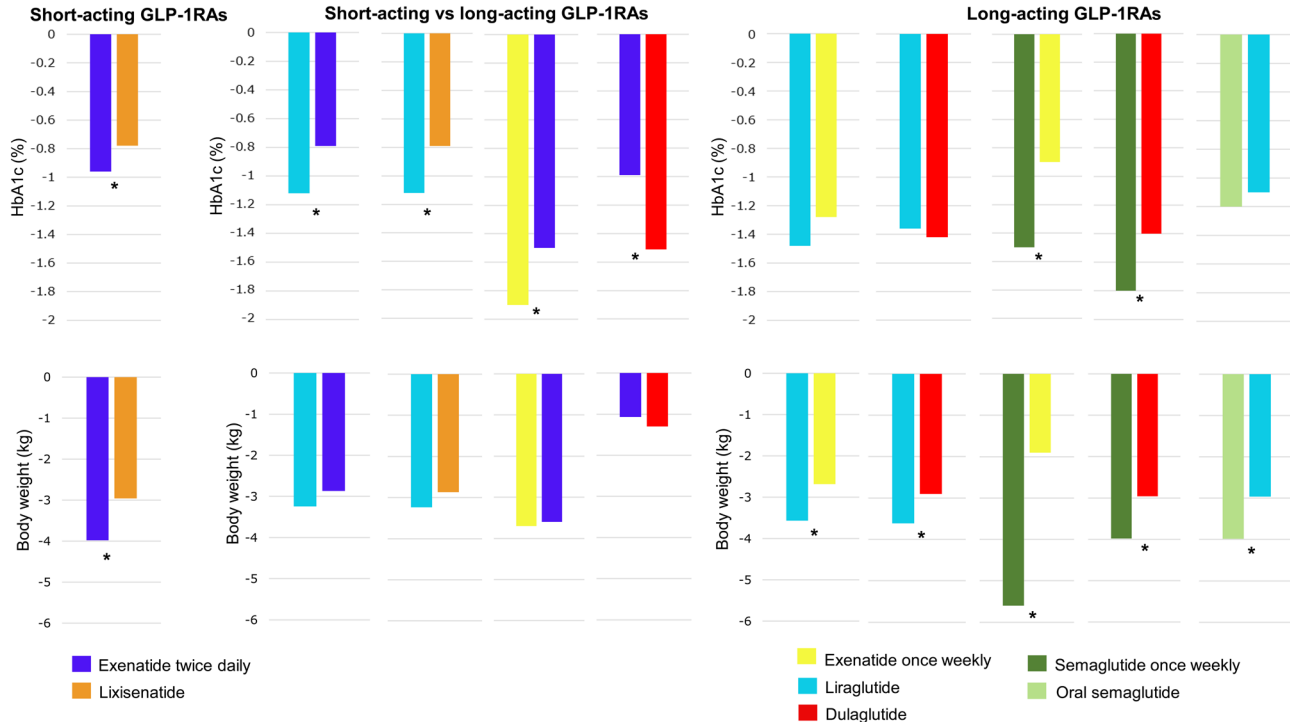
### Long-acting GLP-1RAs

The long-acting GLP-1RAs are exenatide extended-release (once weekly), liraglutide (once daily), dulaglutide (once weekly) and semaglutide (once weekly injections or once daily oral administration). The long-acting GLP-1RAs have been altered in different ways to prolong plasma drug half-life. These modifications include attachment of a fatty acid chain that aids reversible binding to albumin (liraglutide and semaglutide) and covalent binding to carrier molecules such as antibody fragment (Fc) domains of immunoglobulin G (dulaglutide) (11, 12) (Table 1). Exenatide once weekly contains the same active drug as exenatide twice daily, but it is encapsulated in dissolvable microspheres (13). Oral semaglutide is a combination of semaglutide and the absorption enhancer sodium N-(8-(2-hydroxybenzoyl) amino) caprylate (SNAC), which protects semaglutide from degradation in the stomach and facilitates the absorption of semaglutide across the gastric mucosa (14).

### Effects of GLP-1RAs in type 2 diabetes

#### Glycaemia and body weight

All available GLP-1RAs improve glycaemia and have the ability to reduce body weight (15). An overview of the published head-to-head trials that directly compare different pairs of GLP-1RA with the effects on glycaemia and body weight is provided in Fig. 2. The overall glucose-lowering effects, reflected by HbA1c, are generally more pronounced with long-acting GLP-1RAs compared to short-acting GLP-1RAs (Fig. 2). The reduction in HbA1c ranges between 0.8% and 1.8% across trials. As a class, the GLP-1RAs have all been shown to have a body weight-reducing effect without clinically significant differences in body weight reduction between short-acting and long-acting GLP-1RAs. However, there is a large variety in the effects of GLP-1RA treatment between individuals on body weight from weight gain (few) to more than 15% reduction in body weight (16). In most individuals, GLP-1RA-induced body weight loss is achieved within the first 3–6 months of treatment, and in most patients, the body



**Figure 2**

Results from clinical trials comparing the GLP-1 receptor agonists (GLP-1RAs) head-to-head in phase III trials. Left panels are trials comparing short-acting GLP-1RAs (97). Middle panels are trials comparing a short-acting GLP-1RA with a long-acting GLP-1RA (7, 98, 99, 100). Right panel are trials comparing long-acting GLP-1RAs (101, 102, 103, 104, 105, 106). Asterisk (\*) represents significant differences when comparing the GLP-1RAs.

weight loss is maintained if treatment is continued, while discontinuation will lead to some regain of lost body weight. The body weight-reducing effects of the GLP-1RAs in persons with T2D range between 0.3 and 6.3 kg, with the most marked effect observed with dulaglutide (17) and semaglutide (18).

GLP-1RA-induced reduction in body weight is caused by the suppression of food intake (2). In preclinical studies, peripherally administered GLP-1RA reaches and binds to GLP-1Rs in areas of the brain involved in the regulation of food intake in the hypothalamus and hindbrain (2). Peripherally administered GLP-1RA may also indirectly act on the brain by binding to GLP-1Rs on vagal afferent parasympathetic nerve endings, thereby generating and transmitting satiety signals to the hypothalamus and hindbrain (19). In rodents, peripherally administered semaglutide can modulate food preferences by modifying the reward system through dopamine changes in the brain (20). In line with this, a study including patients with obesity randomised to treatment with semaglutide had fewer food cravings and could endure food cravings better than during treatment with placebo (21). The multiple ways to affect the brain and modulate eating behaviour seem to explain the differences observed in body weight reductions between GLP-1RA compounds. Thus, smaller molecules have more accessible entry to penetrate the brain, and long-acting GLP-1RAs are capable of maintaining sufficient plasma levels throughout the day.

### GLP-1RAs and CVOTs

Available GLP-1RAs (except for exenatide twice daily) have been examined in multicentre, double-blinded, randomised, placebo-controlled cardiovascular (CV) outcomes trials (CVOTs). In these trials, participants were randomised to the GLP-1RA in question or placebo, and all participants were treated with standard of care with the possibility of adding non-incretin-based diabetes therapies. Currently, seven CVOTs investigating the CV safety of GLP-1RAs in patients with or without established CVD have been published. As mentioned, albiglutide is no longer available, and thus, details on the HARMONY trial are not included in this clinical review. Baseline participant characteristics and primary composite CV endpoints of the remaining six completed CVOTs for GLP-1RA are listed in Table 2. In the majority of the trials, the primary composite CV endpoint was a three-component major adverse CV event (MACE) outcome including CV-related mortality, non-fatal myocardial infarction (MI), and non-fatal stroke (the CVOT investigating lixisenatide, the ELIXA trial,

**Table 2** Summary of baseline characteristics and primary composite cardiovascular outcomes of the completed CVOTs for GLP-1RAs.

Trial name	Trial drug	Year of completion	N	Median follow-up (years)	Dose/interval	Exposition of drug in trial (%)	Risk factors at baseline					Main outcomes						
							Age (years)	HbA1c (%)	Baseline BMI	% with CVD	% with heart failure	% of statin use	MACE (HR (95% CI))	All-cause mortality (HR(95% CI))	CV mortality (HR(95% CI))	Non-fatal MI (HR(95% CI))	Non-fatal stroke (HR (95% CI))	Time to first occurrence of hospitalisation for UAP (HR (95% CI))
ELIXA (107)	Lixisenatide once daily	2015	6068	2.1	20 µg/day	90.5	60	7.7	30.1	100	22.5	93	1.02 (0.89–1.17)	0.94 (0.78–1.13)	0.98 (0.78–1.22)	1.03 (0.87–1.22)	1.12 (0.79–1.58)	1.11 (0.47–2.62)
LEADER (108)	Liraglutide once daily	2015	9340	3.8	1.8 mg/day	84	64	8.7	32.5	81.3	17.9	72	0.89 (0.78–0.97)	0.85 (0.74–0.97)	0.78 (0.66–0.93)	0.86 (0.73–1.00)	0.86 (0.71–1.06)	
SUSTAIN-6 (49)	Semaglutide once weekly	2016	3297	2.1	0.5/1.0 mg/week	86.5	65	8.7	32.8	80	23.1	73	0.74 (0.58–0.95)	1.05 (0.74–1.50)	0.98 (0.65–1.48)	0.81 (0.57–1.16)	0.65 (0.41–1.03)	
EXSCAL (109)	Exenatide weekly	2017	14,752	3.2	2 mg/week	76	62	8	31.8	73.1	15.8	74	0.91 (0.83–1.00)	0.86 (0.77–0.97)	0.88 (0.76–1.02)	0.97 (0.85–1.10)	0.85 (0.70–1.03)	
REWIND (110)	Dulaglutide once weekly	2019	9901	5.5	1.5 mg/week	82.2	66	7.3	32.3	31.4	8.6	66	0.88 (0.79–0.99)	0.90 (0.80–1.01)	0.91 (0.78–1.06)	0.96 (0.79–1.15)	0.76 (0.62–0.94)	
PIONEER-6 (111)	Oral semaglutide	2019	3183	1.3	14 mg/day	75	66	8.2	32.3	84.6	NR	85	0.79 (0.47–1.11)	0.51 (0.31–0.84)	0.49 (0.27–0.92)	1.04 (0.66–1.66)	0.76 (0.37–1.56)	

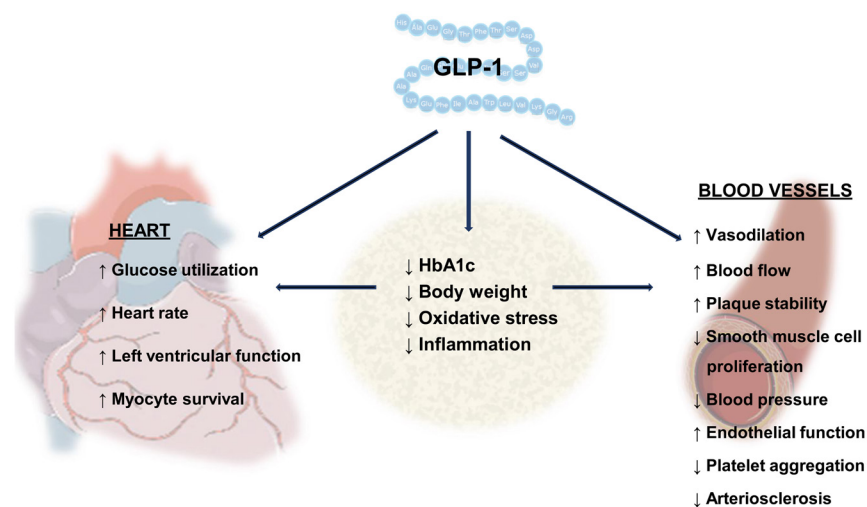
CVD, cardiovascular disease; GLP-1, glucagon-like peptide-1; GLP-1RAs, glucagon-like peptide-1 receptor agonists; HR, hazard ratio; N, number; NR, not reported; UAP, unstable angina pectoris.

also included time to the first occurrence of hospitalisation for unstable angina pectoris). The CVOTs investigating liraglutide (LEADER), s.c. semaglutide (SUSTAIN-6) and dulaglutide (REWIND) have shown significant GLP-1RA-induced reductions in MACE. In the ELIXA trial, no effect of lixisenatide on MACE was observed; perhaps explained by lixisenatide's short half-life compared with the long-acting GLP-1RAs being tested in the other CVOTs (Table 1), the short follow-up period of 2 years and the highest percentage of participants on statin therapy (Table 2). The EXSCEL trial did not find any significant difference in the primary outcome between treatment with the long-acting GLP-1RA exenatide once weekly and placebo. This may be explained by a relatively low trial drug exposure (76%) compared to other CVOTs, and that the trial had no run-in period and, therefore, had one of the highest discontinuation rates. Also, the PIONEER-6 showed no treatment effect with oral semaglutide on the primary composite outcome, which seems to be explained by the low power and short duration of PIONEER-6 (1.3 years). Oral semaglutide demonstrated a hazard ratio (HR) of 0.79, which is like what was observed with s.c. semaglutide in SUSTAIN-6 (HR 0.74). Of note, both PIONEER-6 and SUSTAIN-6 were non-inferiority CV safety studies and, thus, not powered to demonstrate superiority. The larger and longer-lasting (5 years) SOUL trial is ongoing and will determine if oral semaglutide has CV benefits in patients with T2D and CVD or chronic kidney disease (22). The REWIND study found CV benefits of treatment with dulaglutide in participants where only 32% had underlying CVD and who had low baseline HbA1c levels, thereby classifying the study population as a low-risk population compared to the other GLP-1RA CVOTs. A recent meta-analysis including all seven GLP-1RA CVOTs, that is, 42,920 patients, showed a 12%

relative risk reduction in MACE during GLP-1RA treatment compared to placebo. Also, significant reductions in all of the separate components of MACE were observed (15). In addition, treatment with GLP-1RAs reduced all-cause mortality by 12% and hospital admission for heart failure (HF) by 9% compared with placebo. Treatment with GLP-1RA also led to reductions in systolic blood pressure and body weight as well as a slight, sustained increase in heart rate compared to the control arm (15). Another meta-analysis, including 35 trials with GLP-1RAs investigating changes in lipid profile, found that treatment with GLP-1RAs leads to minor improvements in lipid profile and a modest effect on lipid metabolism (23).

### Cardioprotective mechanisms of action

The mechanisms behind the cardioprotective properties of GLP-1RAs are still unclear. Studies have demonstrated that GLP-1RAs exert a direct effect on the heart and blood vessels (6) and an indirect effect via improved glycaemia (24), a reduction in blood pressure and postprandial rise in triglycerides (25), a reduction in markers of oxidative stress and systemic inflammation (26, 27) (Fig. 3). The reduction in MACE observed in treatment with GLP-1RAs is mainly driven by a significant reduction in fatal or non-fatal stroke, but also a significant reduction in risks for CV death and fatal or non-fatal myocardial infarction (15), suggesting that GLP-1RAs may exert their cardioprotective effects through anti-atherosclerotic mechanisms. The atherosclerotic process is complex and strongly associated with inflammation. Treatment with oral semaglutide led to a greater reduction in the inflammatory marker C-reactive protein compared to the sodium-glucose co-transporter-2 inhibitor (SGLT-2i) empagliflozin, without any difference



**Figure 3** Possible cardiovascular actions of glucagon-like peptide 1 (GLP-1) and GLP-1 receptor agonists (GLP-1RAs).

in reduction in body weight between compounds (28). Findings from animal studies suggest that GLP-1R activation may promote anti-arteriosclerotic mechanisms inducing plaque stabilisation and preventing plaque progression (29, 30, 31, 32, 33). Interestingly, GLP-1RA treatment is associated with small reductions in systolic blood pressure, which seem independent of GLP-1RA-induced weight loss (34). This effect is thought to be mediated by increasing natriuresis (35) and vasorelaxation in the renal vasculature (36). In addition, GLP-1RAs increase heart rate in humans, most likely via the GLP-1R located in the sinoatrial node of the heart (37) and perhaps also via activation of the sympathetic nervous system and inhibition of the parasympathetic nervous system as shown in animals studies (38, 39). The increase in heart rate observed in patients treated with GLP-1RAs has led to cautious use of GLP-1RAs in patients with congestive HF New York Heart Associations (NYHA) class IV. In the abovementioned meta-analysis by Kristensen *et al.* (15), a sub-analysis found that GLP-1RAs are safe in patients with HF and in fact reduces their risk of hospitalisation for HF.

### Renal complications

Diabetic kidney disease is a complication of diabetes that leads to increased mortality (40). Most GLP-1RA CVOTs incorporated secondary exploratory renal endpoints. In their meta-analysis, Kristensen *et al.* found that treatment with GLP-1RA leads to a 17% reduction in a broad composite kidney endpoint (development of new-onset macroalbuminuria, doubling of serum creatinine or 40% greater decline in estimated glomerular filtration rate (eGFR), progression to end-stage kidney disease, or kidney-related death), mainly as a result of a reduction in new-onset macroalbuminuria (15). Ongoing trials will determine if liraglutide (41), lixisenatide (ELIXIRS trial) (42) and semaglutide (FLOW trial) (43) reduce dialysis rates and reduce worsening kidney function in people with diabetic kidney disease. The potential nephroprotective mechanisms of GLP-1RAs likely involve GLP-1RA-mediated improvements of conventional risk factors for diabetic kidney disease (control of glycaemia, postprandial lipid level, body weight and blood pressure), but perhaps also direct effects on the kidneys. The exact anatomical location of the GLP-1RA in the human kidney is debated, but the consent is that the GLP-1R is expressed in the renal afferent arterioles, whereas the presence of GLP-1Rs in the renal glomeruli and proximal tubuli remains uncertain (44, 45). Chronic administration of GLP-1RAs have been suggested to affect renal haemodynamic by decreasing eGFR and

ameliorating glomerular hyperfiltration in patients with T2D. In addition, GLP-1RAs may have anti-albuminuria actions by increasing natriuresis and decreasing plasma renin activity (46). In addition, the positive effects of GLP-1RA on oxidative stress and inflammation may also contribute to the nephroprotective mechanisms of GLP-1RAs (47, 48).

### Ophthalmic complications

The ELIXA, EXSCCEL, HARMONY outcomes and PIONEER-6 trials did not include retinopathy as an outcome. In the SUSTAIN-6 trial, there was a significantly higher event rate of diabetic retinopathy in the semaglutide-treated group compared with the control (49). During the first month of the trial, most events coincided with a rapid and massive drop in HbA1c in dysregulated patients with pre-existing retinopathy. A recent CVOT meta-analysis showed that the magnitude of HbA1c reduction was correlated with retinopathy risk and was not associated with GLP-1RA treatment *per se* (50). However, a direct and unknown action of GLP-1RAs cannot be excluded. The ongoing FOCUS trial investigates the long-term effects of semaglutide compared with placebo on diabetic retinopathy, including relatively well-regulated (HbA1c 7–10%) patients with T2D (51).

## Treatment with GLP-1RAs for other indications than T2D and obesity

### Liver diseases

Non-alcoholic fatty liver disease (NAFLD) including non-alcoholic steatohepatitis (NASH) is highly prevalent in patients with T2D and/or obesity. Currently, there are no FDA-approved treatments for NASH. In a recent phase II trial, patients with liver biopsy-confirmed NASH and liver fibrosis were treated once daily with 0.4 mg s.c. semaglutide for 72 weeks. A higher percentage of patients treated with semaglutide had resolution of NASH than with placebo; however, no improvement in fibrosis was found (52). These results are in line with previous findings on the effect of GLP-1RAs on NASH (53, 54). As the GLP-1R is not expressed on human hepatocytes, the GLP-1RA-mediated effects on fatty liver are probably indirectly mediated through GLP-1RA-induced weight loss and ensuing improvement in insulin resistance, and, potentially, anti-inflammatory effects associated with GLP-1RA treatment (29). Integrating the actions of GLP-1 with other receptor agonists is emerging as a potential new drug class with pharmaceutical potential in treating, for example,

liver diseases. The potential of GLP-1/glucagon dual receptor agonist on steatohepatitis, fibrosis and liver regeneration has also shown some promising results in clinical studies (55) and may have a therapeutic potential in NAFLD. In addition, combining semaglutide with cilofexor, a farnesoid X receptor agonist, and/or firsocostat, an acetyl-CoA carboxylase inhibitor, has also shown potential in preclinical studies. Unpublished results from the first phase II trial with the triple combination of semaglutide, cilofexor and/or firsocostat treatment for 24 weeks in 108 patients with NASH showed significant improvements in hepatic steatosis (measured with MRI) and liver injury (measured by serum alanine aminotransferase) in the combination treatment arm vs semaglutide monotherapy (56).

### Type 1 diabetes

By exploiting GLP-1's glucose-dependent glucagonostatic effect and its inhibiting effect on gastric emptying, treatment with GLP-1RAs could supplement insulin treatment in patients with type 1 diabetes (T1D). Liraglutide (57, 58, 59) and short-acting exenatide (60) have been studied in placebo-controlled trials as an add-on to treatment with insulin in patients with T1D. Liraglutide significantly reduced HbA1c and body weight but caused more hypoglycaemic incidents and a slight increase in ketoacidosis in one trial (57). Treatment with short-acting exenatide leads to a minor reduction in HbA1c and significantly reduced body weight without increasing hypoglycaemia or ketoacidosis (60). Thus, no convincing data support the general use of GLP-1RAs as adjuvant therapy in patients with T1D. Still, with the right combination and timing with insulin, treatment with GLP-1RAs may have the potential of reducing HbA1c, postprandial glycaemic excursions, insulin dose, body weight, and frequency of hypoglycaemic episodes, especially in obese patients with T1D (61).

### Neurodegenerative diseases

T2D is a risk factor for cognitive dysfunction and for developing Alzheimer's disease (62). In rodents, modulation of GLP-1R activity can influence amyloid-beta peptide aggregation in Alzheimer's disease (63) and dopamine levels in Parkinson's disease (64). Impaired insulin signalling has been proposed to contribute to the development of Alzheimer's disease (65). GLP-1RAs have shown some neuroprotective effects in rodents with neurodegenerative diseases (65). In a pilot study, treatment with liraglutide increased glucose utilisation in the brain

as detected by (18)F-fluorodeoxyglucose(<sup>18</sup>FDG)-positron emission tomography (PET) scans (66). However, the study was underpowered to make any conclusions on amyloid plaques and cognitive outcome. A phase II study investigating the efficacy and safety of liraglutide in 200 patients with Alzheimer's disease has recently been completed (67) but remains unpublished. Recently, it was announced that a phase III trial investigating whether oral semaglutide can prevent progression in patients with early Alzheimer's disease is to be initiated in 2021 (68). In Parkinson's disease, treatment with exenatide once weekly for 12 months has improved motor symptoms (69). Currently, two ongoing phase II trials investigate the effect of semaglutide (70) and liraglutide (71) on motor symptom progression. Also, one study is evaluating the effect of exenatide once weekly on Parkinson's disease progression by MRI (72). Even though clinical trials have shown some positive results on motor function in patients with Parkinson's disease treated with exenatide (73), a recent Cochrane review found 'low-certainty evidence that exenatide improves motor impairment' in patients with Parkinson's disease (74). Further research is needed to elucidate the role of GLP-1RAs in the treatment of neurodegenerative diseases.

### Safety

The most frequently reported side effects of GLP-1RAs are of gastrointestinal (GI) origin, including nausea, vomiting, diarrhoea and obstipation and may be more common in patients with higher age, lower body weight, renal impairment, and if there is a history or currently smoking (75). GI adverse events are usually mild or moderate, dose-dependent, decline with continued treatment and do not affect glycaemic control. GLP-1RA therapy has previously been linked to acute pancreatitis and pancreatic cancer. However, meta-analyses do not support such an association (76, 77), and an increase in amylase and/or lipase levels may be detected (78). When using GLP-1RAs in patients with risk factors for pancreatitis, such as hypertriglyceridaemia or excessive alcohol use, or in persons with previously diagnosed pancreatitis, great caution is recommended. Since the incidence of cholelithiasis and cholecystitis were higher in patients treated with liraglutide (79), concerns about GLP-1RA and gallbladder adverse events have been raised. In the recent STEP 1 trial investigating the body weight-lowering effect of s.c. semaglutide 2.4 mg once weekly in patients with obesity, gallbladder-related disorders (mostly cholelithiasis) were reported in 2.6%



and 1.2% of participants in the semaglutide and placebo groups, respectively (80). Treatment with GLP-1RAs may affect gallbladder motility and thereby prolong gallbladder refilling (81), which perhaps explains the increase in gallbladder adverse events associated with GLP-1RAs.

## Perspectives

Enthused by the results after bariatric surgery where multiple gut hormones are increased (82), peptide multi-agonists are emerging and have shown promising results. By targeting appetite reduction and energy expenditure with dual or triple agonists, it may be possible to pharmacologically mimic the effects seen with bariatric surgery to produce 30–40% body weight reduction, diabetes remission and CV benefits (83, 84). The dual GLP-1R and GIP receptor agonist tirzepatide is administered once weekly and under development for the treatment of T2D, obesity (85) and NASH (86). In a phase II trial, tirzepatide once weekly was superior to dulaglutide in terms of HbA1c reduction and body weight reduction in patients with T2D with safety data for tirzepatide similar to that of GLP-1RAs (87). Results from the phase III trial (SURPASS 2) investigating the effects of 40 weeks of treatment with tirzepatide vs s.c. semaglutide in patients with T2D have recently been announced, with tirzepatide 15 mg reducing HbA1c levels by 2.46% and body weight reduction by 12.4 kg (13.1%), double the body weight reduction compared to those taking semaglutide 1 mg (88). Tirzepatide has been superior in reducing glycaemia and body weight compared to the GLP-1RA drug class, appearing that GIP adds effectiveness to GLP-1 agonism. CV safety of treatment with tirzepatide in patients with T2D compared to dulaglutide is to be investigated in the SURPASS-CVOT phase III clinical trial scheduled to finish in 2024 (89).

In addition, several dual GLP-1R-glucagon receptor co-agonists are under development, and their therapeutic potential for the treatment of T2D and obesity is being investigated in clinical trials (55, 90, 91, 92). Also, the combination of GLP-1RA with cholecystokinin (CCK) and fibroblast growth factor 21 (FGF21) has been studied in preclinical settings (93, 94). CCK has shown potential in reducing body weight in combination with GLP-1RAs in the mouse models (93). FGF21 is a newly discovered metabolic hormone produced in multiple tissues and secreted in the fasting state and regulates metabolic responses in the pancreas, liver and adipose tissues (95). FGF21 has thermogenic and insulin-sensitising effects when combined with a GLP-1RA with elastin-like polypeptide

(ELP) as a flexible linker (94). In a diabetic mouse model, administration of GLP-1RA-ELP-FGF21 reduced glycaemia without incidences of hypoglycaemia and greater body weight reductions compared with the monotherapy or an equimolar mixture of GLP1-ELP and ELP-FGF21 (94). In summary, the combination of GLP-1RAs with other GI peptide receptor agonists appears to amplify the effects of GLP-1R activation, and these combinations are currently being investigated in preclinical and clinical studies, not only for the treatment of T2D but also for the treatment of other metabolic disorders.

## Conclusion

Over the last decades, extensive preclinical and clinical research have revealed the complexity of the (patho-) physiology of GLP-1 in health and disease. This research and knowledge related to the mode of action have been successfully translated into clinical pharmacology and have been and are a game-changer in T2D and, to some extent, in obesity. GLP-1RAs represent an important drug class for the treatment of T2D and offer marked reductions in plasma glucose levels with a low risk of hypoglycaemia, body weight reductions and a reduction in CVD in patients with high CVD risk. Based on the CVOTs, the treatment algorithm of T2D has changed to include GLP-1RAs independent of glycaemia in patients with established CVD or high-risk (96). Ongoing studies explore the potential of CV, renal and neuroprotective benefits of GLP-1RAs and the potential of using GLP-1RAs for other indications, including NASH, neurodegenerative diseases, and T1D. In addition, emerging dual agonists combining the effects of GLP-1RAs with other peptides have shown a huge potential of superior effects on glycaemia and body weight in patients with T2D compared to currently available peptides. Investigating the physiological and cellular interactions of multi-peptide receptor agonist combinations could further elucidate the therapeutic potential as well as minimising unwanted action or adverse events. Future multi-peptide receptor agonist compounds could potentially target selected organ tissues offering an individualised therapy for a variety of diseases.

### Declaration of interest

C R A and A A declare no conflicts of interest. F K K has served on scientific advisory panels and/or been part of speaker's bureaus, served as a consultant to and/or received research support from Amgen, AstraZeneca, Bayer Boehringer Ingelheim, Carmot Therapeutics, Eli Lilly, Gubra, MedImmune, MSD/Merck, Mundipharma, Norgine, Novo Nordisk, Sanofi

and Zealand Pharma. M M R is a minority shareholder of and consultant for Antag Therapeutics, Bainan Biotech and Synklino. T V has served on scientific advisory panels, been part of speaker's bureaus, served as a consultant to and/or received research support from Amgen, AstraZeneca, Boehringer Ingelheim, Eli Lilly, Gilead, Mundipharma, MSD/Merck, Novo Nordisk, Sanofi and Sun Pharmaceuticals. All authors declare that the study was conducted in the absence of any financial or conflicts of interest.

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