The Ascending GLP-1 Road From Clinical Safety to Reduction of Cardiovascular Complications

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Glucagon-like peptide 1 (GLP-1) was originally identified as a gut-derived incretin hormone that lowered glycemia through potentiation of glucose-dependent insulin secretion. Subsequent studies expanded the actions of GLP-1 to include inhibition of glucagon secretion, gastric emptying, and appetite, collectively useful attributes for a glucose-lowering agent. The introduction of GLP-1 receptor (GLP-1R) agonists for the treatment of diabetes was associated with questions surrounding their safety, principally with regard to medullary thyroid cancer, pancreatitis, and pancreatic cancer, yet cardiovascular outcome trials subsequently revealed reductions in rates of stroke, myocardial infarction, and cardiovascular death with a paucity of major safety signals. We discuss the controversies, unanswered questions, and established use of GLP-1R agonists from a mechanistic and clinical perspective. We highlight methods for detection and cellular sites of GLP-1R expression, key uncertainties, recent insights, and experimental caveats surrounding the use of GLP-1R agonists for the treatment of diabetes and the reduction of diabetes-related complications.

Much of our original understanding of glucagon-like peptide 1 (GLP-1) biology stems from studies in obese animals and humans, with and without diabetes, examining the actions of native GLP-1 (1). Notably, the actions of GLP-1 are highly conserved from small animals to primates, enabling the pivotal demonstration that continuous GLP-1 administration for 6 weeks reduced glucose and body weight while improving insulin sensitivity in human subjects with type 2 diabetes (T2D) (2). The finding that native GLP-1 was rapidly cleared by the kidney and highly sensitive to enzymatic inactivation by dipeptidyl peptidase 4 (DPP-4) shifted the focus for clinical development away from native GLP-1 and toward longer-acting degradation-resistant GLP-1 receptor (GLP-1R) agonists (1). The recent findings that some, but not all, GLP-1R agonists reduce the rates of cardiovascular events in humans with T2D has heightened the interest in mechanisms linking GLP-1R activation to cardioprotection. In this Perspective, we discuss the biology of GLP-1 with a view to unifying concepts linking mechanism to clinical observations. Detailed description of the cardiovascular biology of GLP-1 and the results of major clinical trials are highlighted elsewhere (3–7) and not re-reviewed in detail herein.

An important question that has persisted for decades is whether all of the actions ascribed to native GLP-1 and, subsequently, clinically approved GLP-1R agonists are mediated by the single known GLP-1R (8). Indeed, there are dozens of reports describing direct actions for GLP-1 in muscle, adipose tissue, liver, and cell lines in the absence of expression of the canonical GLP-1R. For studies utilizing native GLP-1, some of the biological activities observed may reflect generation of GLP-1(9-36) and GLP-1(28-36). These smaller peptide fragments exhibit pleiotropic biological activities independent of the known GLP-1R (9), best described in liver, heart, and blood vessels, that are mediated in part through changes in mitochondrial activity (10,11). Nevertheless, mice with inactivation of the *Glp1r* fail to respond to GLP-1R agonists with changes in glycemia, insulin secretion, food intake, body weight, heart rate, or reduction in blood pressure (12–14). Hence, the classic cardiometabolic actions ascribed to GLP-1R agonists require preservation of canonical GLP-1R signaling, although very high levels of endogenous GLP-1 metabolites may contribute to cardiometabolic phenotypes observed in subjects with GLP-1–secreting tumors or following bariatric surgery (15). Here, we update our current understanding of GLP-1 action, highlighting mechanisms linking GLP-1R activation with clinical therapeutic benefit.

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THE CARDIOVASCULAR ACTIONS AND SAFETY OF GLP-1 THERAPIES

Extensive preclinical experimentation in normoglycemic and diabetic rodents and the results of smaller clinical studies in humans with and without T2D raised the possibility that pharmacological GLP-1R agonism was cardioprotective in the context of transient ischemia, myocardial infarction, or heart failure (3,16). Indeed, limited human genetic data support this hypothesis. A low-frequency missense variant (Ala316Thr; rs10305492) in the *GLP1R* gene was associated with reduced fasting glucose and protection against heart disease (17). Multiple cardiovascular outcome trials (CVOTs) have been carried out examining the safety of GLP-1R agonists in individuals with T2D and existing cardiovascular disease (CVD) or multiple CVD risk factors. Individual studies to date revealed cardiovascular safety (18), a trend toward reduction in major cardiovascular events (19), or a clear reduction in major adverse cardiovascular events (MACE) such as stroke (20) and cardiovascular death (6,21). Indeed, a meta-analysis of the major CVOTs was consistent with the notion that the GLP-1R agonists class reduces MACE, cardiovascular mortality, and risk for all-cause mortality (6).

The results of these CVOTs reflect important differences in trial design, patient populations, duration of trial, and drug exposure, as well as drug-specific differences in target engagement and duration of GLP-1R activation (Fig. 1). The first CVOT to report results examined the effect of lixisenatide, a once-daily GLP-1R agonist, in a population of 6,068 individuals with T2D and a recent myocardial infarction (MI) or hospitalization for unstable angina within the previous 180 days (18). Notably, the mean entry HbA_{1c} was ~7.6%, 22% of subjects had a prior MI, and the mean follow-up period was 25 months. Of the GLP-1R agonists studied in CVOTs to date, lixisenatide is unique in exhibiting a pharmacokinetic profile that does not extend to sustained 24-h GLP-1R activation and consequently exhibits less efficacy compared with the longer-acting GLP-1R agonists. Importantly, the trial design reflected the need to accrue a sufficient safety database to meet regulatory approval in a timely manner, rather than one optimized for demonstration of the putative cardiovascular benefits of lixisenatide.

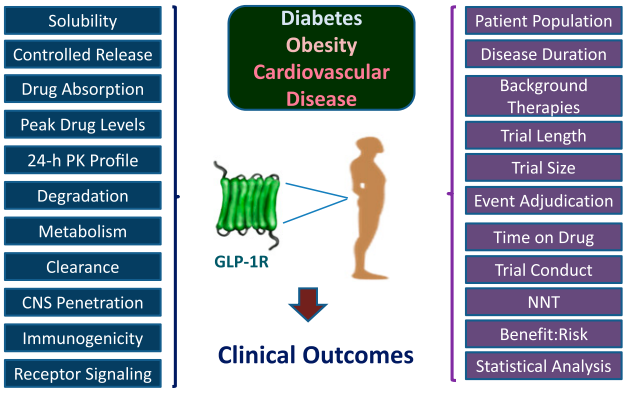

Figure 1— Parameters determining the efficacy of GLP-1R agonists and corresponding clinical trial results. Left panel: Drug-specific differences that contribute to differential GLP-1R activation in target tissues. Right panel: Trial-specific differences that impact clinical trial results examining the efficacy and safety of GLP-1R agonists. CNS, central nervous system; NNT, number needed to treat; PK, pharmacokinetic.

Once-weekly exenatide was developed to provide continuous drug exposure over a 7-day period, enabling sustained engagement of the GLP-1R. Cardiovascular safety was studied in a population of 14,752 subjects with T2D, with HbA_{1c} of 8% at baseline and cardiovascular risk factors, including 73% of subjects with a prior history of CVD (19). Exenatide once-weekly therapy was not associated with a difference in MACE over a mean follow-up period of 3.2 years, yet subjects exhibited a 14% reduction in all-cause mortality (nominally but not statistically significant according to the primary statistical analysis plan hierarchy). Notably, study participants had a mean duration of exposure to the trial regimen of only 2.4 years, with 25% of the study subjects being not fully maintained on the assigned trial regimen and more than 40% of study subjects having prematurely discontinued their assigned study medication (19).

Liraglutide, a small-peptide GLP-1R agonist administered daily, exhibits sustained GLP-1R agonism due to its acylated structure, enabling noncovalent association with albumin and substantial resistance to DPP-4 degradation. The cardiovascular safety of liraglutide was studied in 9,340 subjects with T2D and a mean entry HbA_{1c} of 8.7%, age ≥50 years with known CVD (81%) or ≥60 with one or more CVD risk factors, with a mean duration of drug exposure of 3.5 years. Subjects randomized to liraglutide exhibited fewer MACE events (hazard ratio [HR] 0.87) and a reduced rate of cardiovascular death (HR 0.78).

The cardiovascular safety of semaglutide, an acylated, DPP-4–resistant, long-acting GLP-1R agonist, was studied at two doses, 0.5 or 1.0 mg once weekly, over 104 weeks in 1,638 persons with T2D, 83% with chronic cardiovascular and/or kidney disease (age ≥50) or at least one CVD risk factor (age ≥60), and a mean entry HbA_{1c} of 8.7% (20). Semaglutide-treated subjects were followed for 25 months, with subjects on the drug for ~86.5% of the study period. Semaglutide reduced the rates (HR 0.74) of MACE events (but not cardiovascular death), driven primarily by a reduction in stroke. Notably, HbA_{1c}, body weight, and blood pressure were lower and retinopathy complications were higher in semaglutide-treated patients (20).

As there are no direct head-to-head comparisons of different GLP-1R agonists in randomized controlled CVOTs, it is not possible to attribute differential cardiovascular outcomes obtained with each GLP-1R agonist to the individual properties of the drug versus differences in CVOT design, baseline patient population, and study

execution (Fig. 1). In regard to the mechanistically related yet pharmacologically distinct drug class of DPP-4 inhibitors, these medications exhibit much more similar within-class pharmacokinetics, modest differences in drug metabolism, and fairly comparable degrees of target engagement and pharmacodynamic efficacy (22). The CVOTs for DPP-4 inhibitors have generally yielded similar results, with trial-specific differences in reports of heart failure (4). In contrast, despite the fondness for lumping drugs within a single class together, this tendency makes little sense scientifically for the GLP-1R agonists. These drugs exhibit meaningful differences in structure, dosing regimens, potential for immunogenicity, maximum serum concentration, circulating half-life, and duration of total drug exposure (Fig. 1). These drug-specific properties translate into substantial differences in glycemic and body weight outcomes in head-to-head clinical trials, reflecting differences in the maximal extent and duration of target (GLP-1R) engagement (23). The reduction in MACE events observed for liraglutide and semaglutide is also consistent with the differential metabolic efficacy of these agents, identified in head-to-head studies, relative to other GLP-1R agonists within the class.

Consider a number of attributes and differences for specific drugs within the class. Exenatide once weekly was studied using only two doses, 0.8 and 2.0 mg, in a phase 2 trial involving only 31 subjects with T2D who received exenatide and 12 individuals randomized to placebo over 15 weeks (24). Although the 2.0-mg dose of exenatide once weekly has proved highly effective in multiple clinical trials, it seems likely that a more thorough dose-ranging exploration of its potential therapeutic efficacy might have led to a higher and more efficacious dose being selected for late-stage clinical testing. Similarly, lixisenatide was studied at four different doses—5, 10, 20, or 30 μg once or twice daily—over 13 weeks in subjects with T2D (25). Although the greatest reduction of HbA_{1c} was observed with the 30-μg twice-daily regimen, a dose of 20 μg once daily was selected for clinical development, balancing efficacy versus tolerability; this regimen appeared to produce a smaller reduction in HbA_{1c} compared with twice-daily exenatide in head-to-head testing (26).

In contrast, it is notable that the three most effective GLP-1R agonists—dulaglutide, liraglutide, and semaglutide—were studied more extensively in phase 2 trials, with examination of a broader range of dosing and titration regimens. For example, dulaglutide was initially studied over a range of doses encompassing 0.05, 0.3, 1, 3, 5, or 8 mg once weekly (27), with final dose selection guided in part by an adaptive design integrating reductions in HbA_{1c} balanced by changes in heart rate and blood pressure. Liraglutide was initially studied at daily doses of 0.045, 0.225, 0.45, 0.60, or 0.75 mg (28), followed by subsequent analysis of the effects of 0.6, 1.2, and 1.8 mg daily and ultimately the higher 3.0-mg dose now approved for the treatment of obesity. Semaglutide once weekly, the most recently approved GLP-1R agonist, was studied at doses of 0.1, 0.2, 0.4., 0.8, and 1.6 mg and several different uptitration paradigms (29).

It also remains possible that differences in trial outcomes for GLP-1R agonists, including pharmacodynamic end points such as reduction of glycemia and body weight, reflect less well understood individual drug-specific properties that influence the magnitude and duration of GLP-1R signaling in key target organs (Fig. 1). These may include differential drug transport across vascular beds, differential penetration within the central nervous system, or differential receptor activation, encompassing variability in membrane residency time, biased activation of intracellular signaling pathways, and receptor recycling (30). There has been comparatively little attention paid to whether the clinically approved GLP-1R agonists, which range from small mammalian or lizard-derived peptides to much larger hybrid proteins, exert differential receptor activation in specific cell compartments. Although the current exenatide-based drugs have not yet been shown to clearly reduce MACE events in CVOTs (18,19), there is no evidence that these agents are less effective GLP-1R agonists, relative to human GLP-1R agonists, when tested in GLP-1R signaling studies. Intriguingly, recent experimental evidence from studies of the transferrin receptor indicates the critical importance of ligand size for determining the extent of receptor recycling and, ultimately, expression and signaling at the plasma membrane (31). Whether ligand size constraints impact signaling via the GLP-1R has not been extensively examined.

Hence, the mechanism(s) through which some but not all GLP-1R agonists produce a reduction in rates of MACE remain unclear. It seems reasonable to support a hypothesis that all clinically approved GLP-1R agonists might exhibit similar potential for achieving comparable degrees of cardioprotection, had they each been developed for clinical use based on optimization of dose selection, titration, drug delivery, and receptor signaling, enabling maximal sustained GLP-1R agonism (Fig. 1). Below we discuss existing concepts and unanswered questions surrounding our current understanding of GLP-1 biology in the cardiovascular system.

Detection of GLP-1R Expression

Reliable detection of GLP-1 receptor mRNA and protein in cells and tissues is foundational for understanding GLP-1 action, yet subject to experimental pitfalls. Our laboratory mandates detection of a full-length *GLP1R* mRNA transcript (Fig. 2*A*) prior to assessment of relative expression by quantitative PCR (Fig. 2*B*). Notably, we strive to provide some indication of the abundance of the transcript within a reasonable range of PCR cycle numbers and not just a profile of relative expression (Fig. 2*B* and *C*). Although some newer antibodies for detection of the GLP-1R exhibit reasonable sensitivity and improved specificity (Fig. 2*D*), we have been unable to detect expression of the endogenous GLP-1R (known to be expressed in human islets)

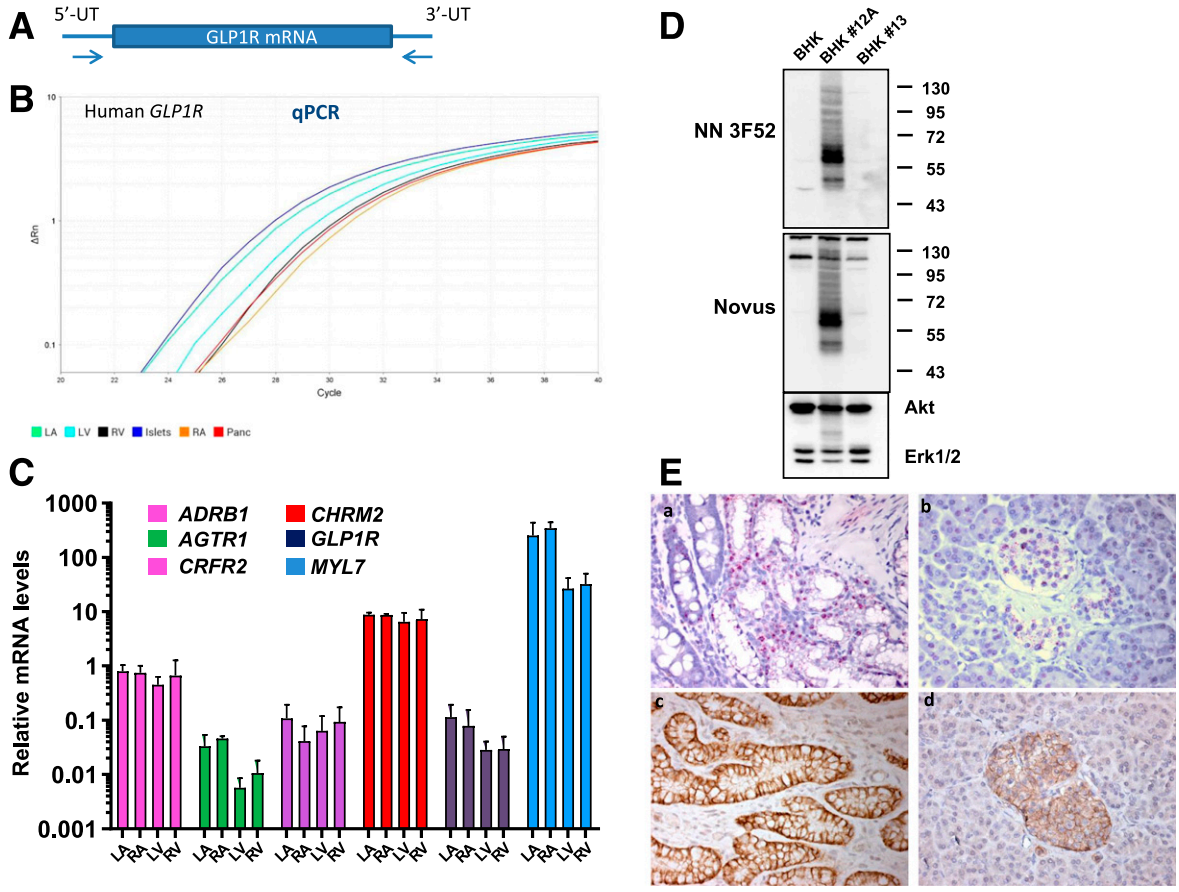

Figure 2—Methods for sensitive and specific detection of GLP-1R expression. *A*: Regular RT-PCR detection of full-length *GLP1R* mRNA transcripts. Ideally, PCR is carried out using primers spanning the entire coding sequence, from 5′-untranslated (UT) to 3′-untranslated regions. *B*: Depiction of relationship between PCR cycle number and relative RNA abundance for analysis of human *GLP1R* expression by quantitative (q)PCR in RNA isolated from left atrium (LA), left ventricle (LV), right ventricle (RV), islets, right atrium (RA), and pancreas (Panc), as described (32). Rn is the fluorescence of the reporter dye divided by the fluorescence of a passive reference dye, and ΔRn is Rn minus the baseline. *C*: Relative RT-PCR quantitation of RNA transcripts for various human receptors, including the *GLP1R*, using RNA isolated from the four different chambers of the human heart, as described (32). *ADRB1*, Adrenoceptor Beta 1; *AGTR1*, Angiotensin II Receptor Type 1; *CRFR2*, Corticotropin-Releasing Factor Receptor 2; *CHRM2*, Cholinergic Receptor Muscarinic 2; *GLP1R*, Glucagon-like Peptide 1 Receptor; *MYL7*, Myosin Light Chain 7. Analysis of pooled RNA samples from subjects 1262, 1371, and 1275, described in ref. 32, is depicted and relative transcript expression normalized to the levels of *EMC7* in each RNA sample. *D*: Western blot analysis (with two different GLP-1R antisera) of the human GLP-1R protein using extracts from baby hamster kidney (BHK) fibroblasts and two BHK clones transfected with a high (BHK #12A) or low (BHK #13) amount of GLP-1R cDNA, as described in and adapted from ref. 32. Molecular size markers in kDa are depicted to the right of the blots. *E*: In situ hybridization (a, b) and immunohistochemistry (c, d) analyses for specific detection of GLP-1R expression from human Brunner's glands (a, c) and human islets (b, d) using reagents and techniques described previously (32).

using conventional Western blot analyses with validated antibodies, likely due in part to low-level expression of class B G-protein–coupled receptors (32). Some antibodies do detect the GLP-1R by immunohistochemistry in Brunner's glands and human islets (Fig. 2*E*); however, careful attention to staining conditions and extensive analysis of positive and negative control tissues is required to mitigate detection of immunopositivity in tissues that do not express the GLP-1R. Despite the improved specificity of monoclonal antibody 3F52 and Novus 19400002, we still detect immunoreactive bands with these antisera using extracts from baby hamster kidney fibroblasts (32). Hence, it seems prudent to use a number of complementary techniques to detect GLP-1R expression with a high degree of sensitivity and specificity.

Blood Vessels

Consider whether and how GLP-1 regulates blood flow, vasodilation, and vascular reactivity in normal or diseased blood vessels. Preclinical experiments in normoglycemic or high-fat diet–fed mice have demonstrated that central nervous system GLP-1R activation controls blood flow in the femoral artery or peripheral tissues, including muscle. Multiple studies have also demonstrated that infusion of native GLP-1 improves flow-mediated vasodilation in humans with type 1 diabetes or T2D, in some settings independent of changes in glucose and insulin (3). These findings understandably raised expectations that GLP-1R agonists might similarly improve endothelial function in human subjects with T2D. Indeed, several studies reported favorable effects of GLP-1R agonists on

flow-mediated vasodilation yet often failed to control for simultaneous changes in glucose, insulin, or body weight arising following acute or chronic administration of GLP-1R agonists in the setting of T2D. Despite reports of GLP-1R expression in endothelial cell lines and endothelial cells propagated ex vivo, it remains difficult to find convincing experimental evidence for expression of the canonical GLP-1R in endothelial cells or vascular smooth muscle cells (VSMs) within major blood vessels, including resistance arteries from rodents or humans in vivo (3,33). Richards et al. (34) detected expression of a reporter gene under the control of *Glp1r* regulatory sequences in mouse aorta and smaller arteries in a subset of VSMs. Perhaps not surprisingly, several carefully controlled studies using degradation-resistant GLP-1R agonists such as exenatide and liraglutide have failed to demonstrate improvement of microvascular endothelial function in obese or nonobese human subjects with T2D (35–37). Moreover, there has been little systematic analysis of GLP-1R expression in endothelial cells or VSMs within major blood vessels from older human subjects with T2D with or without atherosclerosis. Hence, the extent to which GLP-1R agonists might exert direct actions within specific vascular beds requires more investigation.

Blood Pressure

Understanding how GLP-1R agonists reduce blood pressure (BP) independent of weight loss remains enigmatic. Extensive clinical analyses indicate that these agents lower systolic BP in hypertensive subjects with T2D in the presence of coadministered agents such as insulin and metformin (33,38); however, the precise mechanisms and cellular sites linking GLP-1R agonism to reduction of BP in human subjects remain unclear. Intriguingly, GLP-1 does not appear to lower blood pressure in normotensive subjects and GLP-1R agonism has not been associated with the development of hypotension. Indeed, acute administration of native GLP-1 or exenatide attenuated the development of postprandial hypotension in older subjects or individuals with T2D, likely via reduction in the rate of gastric emptying (39). Although weight loss often accompanies and may appear to mirror reductions of BP in trials of GLP-1R agonists, the presence or absence of modest weight loss does not invariably correlate with changes in BP in subjects treated with GLP-1 therapies (40,41). Moreover, changes in systolic BP were modest in CVOTs in subjects treated with liraglutide (1.3 mm lower in LEADER [Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results]) (21) or exenatide (1.57 mm lower in EXSCEL [Exenatide Study of Cardiovascular Event Lowering]) (19) and were unlikely to have accounted for the reductions in mortality reported with liraglutide and exenatide, respectively (19,21). This small additional reduction in BP with GLP-1R agonists observed in controlled clinical trials likely reflects, in part, the extensive use of concomitant BP-lowering medications to achieve BP targets in subjects with diabetes treated within CVOTs and a relatively greater rate of introduction of additional BP medications in the placebo arms of some CVOTs (19,21).

Acute GLP-1 infusion produces a natriuresis in human subjects with T2D or obesity, but the magnitude of any sustained effect in subjects with T2D is modest (42) and appears attenuated over time (43,44). Understanding the mechanisms linking GLP-1R activation to acute or chronic changes in urine sodium excretion is challenging. Although GLP-1 rapidly increases atrial natriuretic factor secretion from the heart in hypertensive rats and mice (14), the majority of hypertensive subjects with diabetes treated acutely or for several weeks with GLP-1R agonists do not exhibit increased plasma levels of atrial natriuretic factor (43). Within the mouse, rat, and human kidney, the GLP-1R has been localized to a few scattered VSMs (34,45,46), and in some instances, colocalized with juxtaglomerular cells. Nevertheless, plasma renin activity is usually not altered in subjects treated with GLP-1R agonists, and there is little evidence from human studies linking sustained changes in the activity or circulating components of the renin angiotensin system with reduction in BP in hypertensive human subjects treated with GLP-1R agonists. Hence, it remains unclear how native GLP-1 and GLP-1R agonists enhance sodium excretion or lower BP in hypertensive animals and humans.

Plasma Lipid Profiles

GLP-1R agonists acutely lower postprandial plasma triglycerides in individuals without diabetes or in subjects with T2D, and LDL levels also trend lower after weight loss in patients with diabetes or obese individuals. The most notable weight-independent actions of GLP-1 on lipids converge on the enterocyte, the cell type responsible for lipid absorption and chylomicron assembly and secretion. Activation of GLP-1R signaling rapidly lowers plasma levels of ApoB48, chylomicrons, and triglycerides in small animals as well as in normoglycemic human subjects or those with diabetes, independent of changes in plasma insulin levels (3,47,48). Moreover, the rapid suppression of postprandial chylomicron and triglyceride levels is independent of gastric emptying (48) and is sustained with prolonged GLP-1R agonism in humans (49). Nevertheless, within the gastrointestinal tract of rodents and humans, the GLP-1R is predominantly localized to enteric neurons and lymphocytes and not detected within enterocytes (50). Hence, the suppression of enterocyte chylomicron secretion observed with GLP-1R agonists is likely indirect, perhaps mediated by neural signals. Moreover, the importance of changes in postprandial lipids as mediators of the cardiovascular actions of GLP-1R agonists or as independent predictors of cardiovascular risk remains unclear.

Inflammation and Hematopoietic Cells

The potential for GLP-1R agonists to modify inflammation might contribute to improved outcomes after MI, reduced plaque rupture and lower rates of stroke, or attenuated development of atherosclerosis (3). Acute or chronic administration of GLP-1R agonists reduces circulating levels

of several inflammatory markers in some but not all human studies of individuals with obesity or T2D (3). GLP-1R expression has been reported in immune cell populations such as macrophages, but rigorous demonstration of the presence of the canonical full-length *GLP1R* mRNA transcript or protein in tissue macrophages from animals or humans, including within diseased blood vessels, is lacking (3,51,52). The available evidence, from publicly available transcriptome analyses such as the ImmGen (Immunological Genome) Project or from experimental studies in mice, localizes functional immune cell GLP-1R expression to a subset of intestinal intraepithelial lymphocytes (53). Similar challenges relate to interpretation of reports linking GLP-1R signaling to inhibition of platelet aggregation and reduced thrombosis in mice (54); notably, it is not yet clear from extensive transcriptomic analyses whether the canonical GLP-1R is expressed, detectable, and functional in mature platelets. Hence, more studies are needed of cells and tissues from animals and humans, ideally with experimental and clinical atherosclerosis and vascular dysfunction, to examine whether and how GLP-1 directly regulates immune cells or platelet biology through expression of the canonical GLP-1R.

Weight Loss

GLP-1R agonists differ widely in their ability to produce weight loss in obese animals and humans; these drug-specific differences likely reflect differential engagement of the GLP-1R in the central nervous system (Fig. 1), where multiple GLP-1R+ regions in the mouse brain transduce signals leading to reduced appetite and weight loss (33,55). It remains unclear whether larger GLP-1R agonists, such as albiglutide and dulaglutide, exhibit the same potential for generating weight loss relative to smaller peptides, perhaps reflecting differential access to populations of GLP-1R+ neurons within brain regions beyond the blood-brain barrier.

Direct GLP-1 Action in the Heart

Observations from multiple laboratories demonstrated that GLP-1R agonists reduce infarct size in preclinical studies (3), either in animals treated with various GLP-1R agonists in vivo or in direct studies of the perfused heart ex vivo. Indeed, transient infusion of exenatide for 6 h at the time of revascularization modestly reduced infarct size in human subjects with or without T2D. Exenatide improved the salvage index based on the ventricular area at risk, as assessed by MRI 90 days after hospitalization for ST-segment elevation MI (56). Although liraglutide administration for several days reduced infarct size and rates of death in normal or diabetic mice (57), the cardioprotective mechanism(s) remain elusive. Findings of reduced numbers of total (fatal and nonfatal) MIs reported in liraglutide-treated subjects in LEADER (21) has heightened interest in identification of mechanisms linking GLP-1R signaling to ischemic cardioprotection. Although some studies demonstrate that GLP-1R agonism directly modulates cardiac fuel metabolism in normal or dysfunctional hearts from human subjects with or without T2D, the existing human data are inconclusive (58,59), and limited information is available regarding whether and how GLP-1 alters myocardial fuel metabolism in the ischemic or failing human heart. The accumulated evidence suggests that the GLP-1R is predominantly expressed in atria, and not ventricles, in hearts from mice and rats (3), indirectly localized via reporter gene expression to atrial cardiomyocytes and VSMs in mouse ventricle (34). Subsequent studies identified GLP-1R expression within the sinoatrial node as detected by immunocytochemistry, ligand binding, and in situ hybridization in monkeys and humans (32,45).

Indeed, GLP-1R agonists consistently increase heart rate in human subjects who are normoglycemic, obese, or have diabetes, and the relative increase in heart rate is likely proportional to the extent and duration of GLP-1R engagement (Fig. 2) (38). Although conditional genetic reduction in expression of the atrial GLP-1R did not diminish the cardioprotective actions of liraglutide in mice, heart rate was reduced in animals following reduction of atrial GLP-1R expression (60). Hence, although the GLP-1R–dependent increase in heart rate likely reflects contributions from engagement of the GLP-1R within the autonomic nervous system and sinoatrial node, the mechanisms and cell types linking GLP-1R agonism to reduction of infarct size in preclinical studies are not known.

Even less data informs the expression and localization of the GLP-1R within the human heart. Wallner et al. (61) detected *GLP1R* mRNA transcripts by RT-PCR in RNA isolated from all four chambers of the human heart, including right and left ventricles, as well as in isolated human cardiomyocytes from the left ventricle (61). Consistent with these findings, full-length *GLP1R* mRNA transcripts were detected in RNA isolated from all four chambers of 15 human hearts (Fig. 2) at levels approximating those detected in human pancreas (32). Nevertheless, the results of these studies reflect the very small areas of heart sampled in each biopsy and may not be indicative of *GLP1R* expression within different regions of each heart chamber. Moreover, the specific cell types within the heart containing *GLP1R* mRNA transcripts or immunoreactive GLP-1R protein were not detected following analysis of sections from several dozen human ventricles (32). Hence, the precise cellular localization of human cardiac GLP-1R expression requires further study.

Heart Failure

Preliminary studies using native GLP-1 in dogs and humans suggested that continuous GLP-1R agonism improves ventricular function in the setting of chronic heart failure (3). The interpretation of these findings was subsequently challenged by data recapitulating the beneficial action of native GLP-1 using infusion of GLP-1(9-36), a poor agonist at the GLP-1 receptor, in dogs with pacing-induced heart failure (62). Reassuringly, no heart

failure–related adverse outcomes were detected in studies examining the safety of GLP-1R agonists in the Evaluation of Lixisenatide in Acute Coronary Syndrome (ELIXA), LEADER, Trial to Evaluate Cardiovascular and Other Long-term Outcomes With Semaglutide in Subjects With Type 2 Diabetes (SUSTAIN-6), or EXSCEL (18–21), which collectively enrolled thousands of subjects with mild to moderate heart failure. Nevertheless, three smaller dedicated studies of more advanced heart failure, two with liraglutide (24 weeks) and one with albiglutide (12 weeks), failed to demonstrate functional improvement in human subjects, with or without T2D, with reduced ejection fraction and a history of hospitalization for heart failure (59,63,64). Indeed, rehospitalization for heart failure was numerically more common with liraglutide (63), and increased reports of arrhythmias, including supraventricular tachycardia, were noted in liraglutide-treated subjects in these trials (63,64), consistent with the localization of GLP-1R expression to the sinoatrial node. Hence, the preclinical promise of GLP-1 as an inotropic agent has not been validated in human studies.

The Kidney

CVOTs have reported reduced rates of renal end points with liraglutide and semaglutide but not with exenatide once weekly or lixisenatide (18–21), driven principally by fewer reports of microalbuminuria. Nevertheless, there is no evidence that GLP-1R agonists reduce progression to chronic kidney disease or the time to renal replacement therapy in humans with T2D. How sustained GLP-1R engagement reduces albumin excretion in the diabetic kidney is not known. Although extensive preclinical experimentation in diabetic mice and rats supports the finding of reduced albumin excretion and decreased kidney inflammation following treatment with GLP-1R agonists, there is a lack of consensus as to the prevailing mechanism(s) (33). Notably, the GLP-1R appears predominantly localized to a few scattered VSMs in some but not most blood vessels within the rodent and human kidney, hence the link between systemic GLP-1R activation and reduced excretion of albumin remains uncertain.

The Liver

Administration of GLP-1R agonists also reduces liver fat and inflammation in animal studies and decreases liver injury, inflammation, and fibrosis in human subjects with nonalcoholic steatohepatitis (NASH) (33,65). Of the 23 subjects with NASH treated with liraglutide 1.8 mg daily for 48 weeks and assessed using repeated liver biopsies, 9 experienced resolution of NASH, whereas 2 subjects exhibited progression of fibrosis (65). Nevertheless, the available evidence does not support expression of the canonical GLP-1R within rodent or human hepatocytes, and it remains unclear whether mechanisms beyond weight loss link the therapeutic benefits of GLP-1 with reduction of liver fat and decreased hepatocellular injury and inflammation.

Adverse Events Associated With GLP-1R Agonists

The most common adverse events noted with clinical use of GLP-1R agonists include nausea, vomiting, and diarrhea, likely reflecting central aversive actions of GLP-1 and the GLP-1R–dependent control of gut motility. Regulatory concerns surrounding the risk of thyroid C-cell hyperplasia and medullary thyroid carcinoma (MTC) were based entirely on data from mice and rats, which express the canonical GLP-1R on thyroid C cells, linked to stimulation of calcitonin secretion and C-cell proliferation (66,67). In contrast, it is difficult to detect GLP-1R expression within primate thyroid C cells (67), and sustained GLP-1R agonism does not induce C-cell hyperplasia in monkeys (67,68). Indeed, associated reports of GLP-1R expression within the normal and neoplastic human thyroid (69) reflected the use of nonspecific, incompletely validated GLP-1R antisera not suitable for detection of the GLP-1R (32,51). Subsequent assessment using a validated GLP-1R antibody to analyze 44 different normal and tumorous human thyroid glands did not detect GLP-1R expression by immunocytochemistry in normal thyroid C cells nor in the majority of MTC cases examined (70). Moreover, extensive clinical follow-up coupled with thousands of calcitonin measurements have failed to reveal evidence for a functional GLP-1R C-cell axis or MTC in human subjects with diabetes or obesity treated for several years with long-acting GLP-1R agonists (71,72).

The possibility that therapy with GLP-1R agonists might predispose susceptible individuals to the development of acute or chronic pancreatitis or pancreatic cancer has received considerable scrutiny (73). These concerns initially arose following clinical reports of acute pancreatitis in human subjects treated with exenatide. Simultaneously, histological studies reported GLP-1R expression within pancreatic ducts and preneoplastic lesions in animals and humans, and preclinical experiments linked incretin signaling to pancreatic inflammation and cell proliferation in rodents (74). Notably, efforts to reproduce many of these preclinical findings were unsuccessful, reflecting limitations of many of the reagents and experimental designs used in these studies (74,75). Subsequent examination of a large number of pancreata from different strains of rats revealed spontaneous rates of histological abnormalities, including pancreatitis, in up to 72% of animals never exposed to incretin therapy (76). Although small rises in plasma lipase and, to a lesser extent, amylase have been reported in some human subjects with diabetes treated with GLP-1R agonists, these observations reflect direct activation of the canonical pancreatic acinar cell GLP-1R coupled to enzyme secretion, and not subclinical pancreatitis (33).

The safety of GLP-1R agonists has also been scrutinized in multiple CVOTs, which failed to generate a signal linking sustained therapy with GLP-1R agonists to pancreatitis or thyroid or pancreatic cancer (6). Similarly, in 2,024,441 person-years of follow-up, examination of health records failed to reveal increased reports of pancreatic cancer in individuals treated with incretin-based therapies (77). Given

the long latency required for cancer signals to emerge, coupled with the low incidence rates for malignancies such as MTC, it is not yet possible to completely exclude the possibility that a cancer signal might yet emerge in longer, larger studies or following scrutiny of more extensive drug exposure using electronic health databases.

AREAS OF UNCERTAINTY

The recent observation that therapy with some sodium–glucose cotransporter 2 inhibitors and GLP-1R agonists leads to a reduction in MACE and cardiovascular death has transformed concepts and goals for the treatment of T2D. Nevertheless, the majority of subjects with T2D treated in primary care settings do not resemble the subjects enrolled in these CVOTs; patients may be younger and often have no evidence of established CVD. The available evidence suggests that the HR for MACE in patients without clinical CVD is not reduced, perhaps related to lower event rates, small numbers, and inadequate duration of exposure to GLP-1R agonists. Given the uncertainty about the cardioprotective actions of GLP-1R agonists for primary prevention of CVD, there is insufficient evidence to extrapolate the extraordinary results seen in LEADER and related trials to individuals who do not meet the entry criteria for these trials. It seems unlikely we will obtain new information informing decisions for subjects older than those studied in the CVOTs, individuals with HbA_{1c} less than 7%, or younger individuals not meeting the eligibility criteria for specific CVOTs. Encouragingly, a study examining the cardiovascular safety of investigational semaglutide in individuals without diabetes but with obesity, the SELECT trial (semaglutide effects on cardiovascular outcomes in people with overweight or obesity), is under way. At present, health care providers must use the available evidence base, albeit incomplete, coupled with country-specific guidelines, to advance personalized therapeutic recommendations where the evidence is lacking.

Similarly unclear is whether GLP-1R agonists should be evaluated in selected populations of individuals without diabetes with established cardiovascular disease for purposes of secondary prevention. Given the current uncertainty surrounding mechanisms linking GLP-1 action to reduction of MACE events, it seems prudent to focus on delineation of how GLP-1R signaling controls cardiovascular biology in people with T2D prior to launching new ambitious therapeutic interventions surrounded by questions of mechanistic plausibility. Although sensitive and specific methods are available for cellular and tissue localization of GLP-1R mRNA and protein (Fig. 2), the literature is replete with examples of failure to apply rigorous methodology for detection of GLP-1R expression, leading to erroneous conclusions surrounding the mechanisms of GLP-1 action (75). For example, the observation of numerically greater retinopathy events in SUSTAIN-6 (20) calls into question the putative localization and functional importance of GLP-1R expression in the retina versus the established importance of rapid glucose lowering, which predisposes toward development of retinopathy. Although *GLP1R* mRNA transcripts were reported in the human retina, the relative levels were only slightly higher than those observed in liver, a tissue not known to express the canonical GLP-1R (33,78). Moreover, previous reports localizing immunoreactive GLP-1R protein within the normal or diabetic human retina may be suspect owing to the use of nonvalidated GLP-1R antisera (78). More recent studies using a validated GLP-1R antibody together with in situ hybridization localized the GLP-1R to a few neurons within the ganglion cell layer of the human retina (79); GLP-1R+ cells were not detected within the retinal epithelium or blood vessels in eyes from individuals with history of proliferative diabetic retinopathy.

The general conservation of GLP-1 biology in animals and humans with or without metabolic disease suggests that ongoing analysis of mechanisms of GLP-1 action in preclinical studies remains reasonable, ideally complemented wherever possible by similar studies using human cells and tissues. It is now more than 13 years since the first approval of a GLP-1R agonist for the treatment of T2D, and despite much apprehension about the safety of these agents, the clinical safety database is now considerable and largely reassuring (6). It seems timely to ask whether the agency-mandated warnings surrounding pancreatitis and MTC for GLP-1R agonists do more harm than good, given the associated apprehension versus the actual clinical data from CVOTs and the potential for reduction of MACE and cardiovascular death. The therapeutic benefit of the GLP-1 system for diabetes and obesity is now firmly established, based on a combination of robust basic and clinical science (1,33). Although we still have much to learn, the road ahead is well paved, brightly illuminated, and holds much promise for ongoing innovation in the treatment of metabolic disease.

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References

1. Drucker DJ, Habener JF, Holst JJ. Discovery, characterization, and clinical development of the glucagon-like peptides. J Clin Invest 2017;127:4217–4227

2. Zander M, Madsbad S, Madsen JL, Holst JJ. Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. Lancet 2002;359:824–830

3. Drucker DJ. The cardiovascular biology of glucagon-like peptide-1. Cell Metab 2016;24:15–30

4. Nauck MA, Meier JJ, Cavender MA, Abd El Aziz M, Drucker DJ. Cardiovascular actions and clinical outcomes with glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors. Circulation 2017;136:849–870

5. Kaul S. Mitigating cardiovascular risk in type 2 diabetes with antidiabetes drugs: a review of principal cardiovascular outcome results of EMPA-REG OUTCOME, LEADER, and SUSTAIN-6 trials. Diabetes Care 2017;40:821–831

6. Bethel MA, Patel RA, Merrill P, et al.; EXSCEL Study Group. Cardiovascular outcomes with glucagon-like peptide-1 receptor agonists in patients with type 2 diabetes: a meta-analysis. Lancet Diabetes Endocrinol 2018;6:105–113
7. Andersen A, Lund A, Knop FK, Vilsbøll T. Glucagon-like peptide 1 in health and disease. Nat Rev Endocrinol 2018;14:390–403
8. Thorens B. Expression cloning of the pancreatic β cell receptor for the gluco-incretin hormone glucagon-like peptide 1. Proc Natl Acad Sci U S A 1992;89:8641–8645
9. Abu-Hamdah R, Rabiee A, Meneilly GS, Shannon RP, Andersen DK, Elahi D. Clinical review: the extrapancreatic effects of glucagon-like peptide-1 and related peptides. J Clin Endocrinol Metab 2009;94:1843–1852
10. Ban K, Noyan-Ashraf MH, Hoefer J, Bolz SS, Drucker DJ, Husain M. Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. Circulation 2008;117:2340–2350
11. Giacco F, Du X, Carratú A, et al. GLP-1 cleavage product reverses persistent ROS generation after transient hyperglycemia by disrupting an ROS-generating feedback loop. Diabetes 2015;64:3273–3284
12. Scrocchi LA, Brown TJ, MaClusky N, et al. Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene. Nat Med 1996;2:1254–1258
13. Tatarkiewicz K, Sablan EJ, Polizzi CJ, Villescaz C, Parkes DG. Long-term metabolic benefits of exenatide in mice are mediated solely via the known glucagon-like peptide 1 receptor. Am J Physiol Regul Integr Comp Physiol 2014;306:R490–R498
14. Kim M, Platt MJ, Shibasaki T, et al. GLP-1 receptor activation and Epac2 link atrial natriuretic peptide secretion to control of blood pressure. Nat Med 2013;19:567–575
15. Drucker DJ. Evolving concepts and translational relevance of enteroendocrine cell biology. J Clin Endocrinol Metab 2016;101:778–786
16. Ussher JR, Drucker DJ. Cardiovascular actions of incretin-based therapies. Circ Res 2014;114:1788–1803
17. Scott RA, Freitag DF, Li L, et al.; CVD50 Consortium; GERAD_EC Consortium; Neurology Working Group of the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE); Alzheimer's Disease Genetics Consortium; Pancreatic Cancer Cohort Consortium; European Prospective Investigation into Cancer and Nutrition–Cardiovascular Disease (EPIC-CVD); EPIC-INTERACT; CHARGE Consortium; The CHD Exome+ Consortium; CARDIOGRAM Exome Consortium. A genomic approach to therapeutic target validation identifies a glucose-lowering GLP1R variant protective for coronary heart disease. Sci Transl Med 2016;8:341ra76
18. Pfeffer MA, Claggett B, Diaz R, et al.; ELIXA Investigators. Lixisenatide in patients with type 2 diabetes and acute coronary syndrome. N Engl J Med 2015;373:2247–2257
19. Holman RR, Bethel MA, Mentz RJ, et al.; EXSCEL Study Group. Effects of once-weekly exenatide on cardiovascular outcomes in type 2 diabetes. N Engl J Med 2017;377:1228–1239
20. Marso SP, Bain SC, Consoli A, et al.; SUSTAIN-6 Investigators. Semaglutide and cardiovascular outcomes in patients with type 2 diabetes. N Engl J Med 2016;375:1834–1844
21. Marso SP, Daniels GH, Brown-Frandsen K, et al.; LEADER Steering Committee; LEADER Trial Investigators. Liraglutide and cardiovascular outcomes in type 2 diabetes. N Engl J Med 2016;375:311–322
22. Mulvihill EE, Drucker DJ. Pharmacology, physiology, and mechanisms of action of dipeptidyl peptidase-4 inhibitors. Endocr Rev 2014;35:992–1019
23. Taylor SI. GLP-1 receptor agonists: differentiation within the class. Lancet Diabetes Endocrinol 2018;6:83–85
24. Kim D, MacConell 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;30:1487–1493
25. Ratner RE, Rosenstock J, Boka G; DRI6012 Study Investigators. Dose-dependent effects of the once-daily GLP-1 receptor agonist lixisenatide in patients with type 2 diabetes inadequately controlled with metformin: a randomized, double-blind, placebo-controlled trial. Diabet Med 2010;27:1024–1032
26. Rosenstock J, Raccah D, Korányi 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;36:2945–2951
27. Barrington P, Chien JY, Showalter HD, et al. A 5-week study of the pharmacokinetics and pharmacodynamics of LY2189265, a novel, long-acting glucagon-like peptide-1 analogue, in patients with type 2 diabetes. Diabetes Obes Metab 2011;13:426–433
28. Madsbad S, Schmitz O, Ranstam J, Jakobsen G, Matthews DR; NN2211-1310 International Study Group. Improved glycemic control with no weight increase in patients with type 2 diabetes after once-daily treatment with the long-acting glucagon-like peptide 1 analog liraglutide (NN2211): a 12-week, double-blind, randomized, controlled trial. Diabetes Care 2004;27:1335–1342
29. Nauck MA, Petrie JR, Sesti G, et al.; Study 1821 Investigators. A phase 2, randomized, dose-finding study of the novel once-weekly human GLP-1 analog, semaglutide, compared with placebo and open-label liraglutide in patients with type 2 diabetes. Diabetes Care 2016;39:231–241
30. Wacker D, Stevens RC, Roth BL. How ligands illuminate GPCR molecular pharmacology. Cell 2017;170:414–427
31. DeGroot ACM, Busch DJ, Hayden CC, et al. Entropic control of receptor recycling using engineered ligands. Biophys J 2018;114:1377–1388
32. Baggio LL, Yusta B, Mulvihill EE, et al. GLP-1 receptor expression within the human heart. Endocrinology 2018;159:1570–1584
33. Drucker DJ. Mechanisms of action and therapeutic application of glucagon-like peptide-1. Cell Metab 2018;27:740–756
34. 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;63:1224–1233
35. Kelly AS, Bergenstal RM, Gonzalez-Campoy JM, Katz H, Bank AJ. Effects of exenatide vs. metformin on endothelial function in obese patients with pre-diabetes: a randomized trial. Cardiovasc Diabetol 2012;11:64
36. Nandy D, Johnson C, Basu R, et al. The effect of liraglutide on endothelial function in patients with type 2 diabetes. Diab Vasc Dis Res 2014;11:419–430
37. Nomoto H, Miyoshi H, Furumoto T, et al.; SAIS Study Group. A comparison of the effects of the GLP-1 analogue liraglutide and insulin glargine on endothelial function and metabolic parameters: a randomized, controlled trial Sapporo Athero-Incretin Study 2 (SAIS2). PLoS One 2015;10:e0135854
38. Meier JJ, Rosenstock J, Hincelin-Méry A, et al. Contrasting effects of lixisenatide and liraglutide on postprandial glycemic control, gastric emptying, and safety parameters in patients with type 2 diabetes on optimized insulin glargine with or without metformin: a randomized, open-label trial. Diabetes Care 2015;38:1263–1273
39. Trahair LG, Horowitz M, Stevens JE, et al. Effects of exogenous glucagon-like peptide-1 on blood pressure, heart rate, gastric emptying, mesenteric blood flow and glycaemic responses to oral glucose in older individuals with normal glucose tolerance or type 2 diabetes. Diabetologia 2015;58:1769–1778
40. Ferdinand KC, White WB, Calhoun DA, et al. Effects of the once-weekly glucagon-like peptide-1 receptor agonist dulaglutide on ambulatory blood pressure and heart rate in patients with type 2 diabetes mellitus. Hypertension 2014;64:731–737
41. Katout M, Zhu H, Rutsky J, et al. Effect of GLP-1 mimetics on blood pressure and relationship to weight loss and glycemia lowering: results of a systematic meta-analysis and meta-regression. Am J Hypertens 2014;27:130–139
42. Tonneijck L, Muskiet MHA, Smits MM, et al. Postprandial renal haemodynamic effect of lixisenatide vs once-daily insulin-glulisine in patients with type 2 diabetes on insulin-glargine: an 8-week, randomised, open-label trial. Diabetes Obes Metab 2017;19:1669–1680
43. Lovshin JA, Barnie A, DeAlmeida A, Logan A, Zinman B, Drucker DJ. Liraglutide promotes natriuresis but does not increase circulating levels of atrial natriuretic peptide in hypertensive subjects with type 2 diabetes. Diabetes Care 2015;38:132–139

44. Tonneijck L, Smits MM, Muskiet MH, et al. Renal effects of DPP-4 inhibitor sitagliptin or GLP-1 receptor agonist liraglutide in overweight patients with type 2 diabetes: a 12-week, randomized, double-blind, placebo-controlled trial. Diabetes Care 2016;39:2042–2050
45. Pyke C, Heller RS, Kirk RK, et al. GLP-1 receptor localization in monkey and human tissue: novel distribution revealed with extensively validated monoclonal antibody. Endocrinology 2014;155:1280–1290
46. Jensen EP, Poulsen SS, Kissow H, et al. Activation of GLP-1 receptors on vascular smooth muscle cells reduces the autoregulatory response in afferent arterioles and increases renal blood flow. Am J Physiol Renal Physiol 2015;308:F867–F877
47. Hsieh J, Longuet C, Baker CL, et al. The glucagon-like peptide 1 receptor is essential for postprandial lipoprotein synthesis and secretion in hamsters and mice. Diabetologia 2010;53:552–561
48. Xiao C, Bandsma RH, Dash S, Szeto L, Lewis GF. Exenatide, a glucagon-like peptide-1 receptor agonist, acutely inhibits intestinal lipoprotein production in healthy humans. Arterioscler Thromb Vasc Biol 2012;32:1513–1519
49. Hermansen K, Bækdal TA, Düring M, et al. Liraglutide suppresses postprandial triglyceride and apolipoprotein B48 elevations after a fat-rich meal in patients with type 2 diabetes: a randomized, double-blind, placebo-controlled, cross-over trial. Diabetes Obes Metab 2013;15:1040–1048
50. Wismann P, Barkholt P, Secher T, et al. The endogenous preproglucagon system is not essential for gut growth homeostasis in mice. Mol Metab 2017;6:681–692
51. Panjwani N, Mulvihill EE, Longuet C, et al. GLP-1 receptor activation indirectly reduces hepatic lipid accumulation but does not attenuate development of atherosclerosis in diabetic male *ApoE*$^{-/-}$ mice. Endocrinology 2013;154:127–139
52. Cochain C, Vafadarnejad E, Arampatzi P, et al. Single-cell RNA-seq reveals the transcriptional landscape and heterogeneity of aortic macrophages in murine atherosclerosis. Circ Res 2018:CIRCRESAHA.117.312509
53. Yusta B, Baggio LL, Koehler J, et al. GLP-1 receptor (GLP-1R) agonists modulate enteric immune responses through the intestinal intraepithelial lymphocyte GLP-1R. Diabetes 2015;64:2537–2549
54. Cameron-Vendrig A, Reheman A, Siraj MA, et al. Glucagon-like peptide 1 receptor activation attenuates platelet aggregation and thrombosis. Diabetes 2016;65:1714–1723
55. Burmeister MA, Ayala JE, Smouse H, et al. The hypothalamic glucagon-like peptide 1 receptor is sufficient but not necessary for the regulation of energy balance and glucose homeostasis in mice. Diabetes 2017;66:372–384
56. Lønborg J, Vejlstrup N, Kelbæk H, et al. Exenatide reduces reperfusion injury in patients with ST-segment elevation myocardial infarction. Eur Heart J 2012;33:1491–1499
57. Noyan-Ashraf MH, Momen MA, Ban K, et al. GLP-1R agonist liraglutide activates cytoprotective pathways and improves outcomes after experimental myocardial infarction in mice. Diabetes 2009;58:975–983
58. Gejl M, Søndergaard HM, Stecher C, et al. Exenatide alters myocardial glucose transport and uptake depending on insulin resistance and increases myocardial blood flow in patients with type 2 diabetes. J Clin Endocrinol Metab 2012;97:E1165–E1169
59. Lepore JJ, Olson E, Demopoulos L, et al. Effects of the novel long-acting GLP-1 agonist, albiglutide, on cardiac function, cardiac metabolism, and exercise capacity in patients with chronic heart failure and reduced ejection fraction. JACC Heart Fail 2016;4:559–566
60. Ussher JR, Baggio LL, Campbell JE, et al. Inactivation of the cardiomyocyte glucagon-like peptide-1 receptor (GLP-1R) unmasks cardiomyocyte-independent GLP-1R-mediated cardioprotection. Mol Metab 2014;3:507–517
61. Wallner M, Kolesnik E, Ablasser K, et al. Exenatide exerts a PKA-dependent positive inotropic effect in human atrial myocardium: GLP-1R mediated effects in human myocardium. J Mol Cell Cardiol 2015;89(Pt B):365–375
62. Nikolaidis LA, Elahi D, Shen YT, Shannon RP. Active metabolite of GLP-1 mediates myocardial glucose uptake and improves left ventricular performance in conscious dogs with dilated cardiomyopathy. Am J Physiol Heart Circ Physiol 2005;289:H2401–H2408
63. Margulies KB, Hernandez AF, Redfield MM, et al.; NHLBI Heart Failure Clinical Research Network. Effects of liraglutide on clinical stability among patients with advanced heart failure and reduced ejection fraction: a randomized clinical trial. JAMA 2016;316:500–508
64. Jorsal A, Kistorp C, Holmager P, et al. Effect of liraglutide, a glucagon-like peptide-1 analogue, on left ventricular function in stable chronic heart failure patients with and without diabetes (LIVE)—a multicentre, double-blind, randomised, placebo-controlled trial. Eur J Heart Fail 2017;19:69–77
65. Armstrong MJ, Gaunt P, Aithal GP, et al.; LEAN trial team. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. Lancet 2016;387:679–690
66. Yamada C, Yamada Y, Tsukiyama K, et al. The murine glucagon-like peptide-1 receptor is essential for control of bone resorption. Endocrinology 2008;149:574–579
67. Bjerre Knudsen L, Madsen LW, Andersen S, et al. Glucagon-like peptide-1 receptor agonists activate rodent thyroid C-cells causing calcitonin release and C-cell proliferation [published correction appears in Endocrinology 2012;153:1000]. Endocrinology 2010;151:1473–1486
68. Vahle JL, Byrd RA, Blackbourne JL, et al. Effects of dulaglutide on thyroid C cells and serum calcitonin in male monkeys. Endocrinology 2015;156:2409–2416
69. Gier B, Butler PC, Lai CK, Kirakossian D, DeNicola MM, Yeh MW. Glucagon like peptide-1 receptor expression in the human thyroid gland. J Clin Endocrinol Metab 2012;97:121–131
70. Waser B, Blank A, Karamitopoulou E, Perren A, Reubi JC. Glucagon-like-peptide-1 receptor expression in normal and diseased human thyroid and pancreas. Mod Pathol 2015;28:391–402
71. Hegedüs L, Moses AC, Zdravkovic M, Le Thi T, Daniels GH. GLP-1 and calcitonin concentration in humans: lack of evidence of calcitonin release from sequential screening in over 5000 subjects with type 2 diabetes or nondiabetic obese subjects treated with the human GLP-1 analog, liraglutide. J Clin Endocrinol Metab 2011;96:853–860
72. Hegedüs L, Sherman SI, Tuttle RM, et al.; LEADER Publication Committee on behalf of the LEADER Trial Investigators. No evidence of increase in calcitonin concentrations or development of C-cell malignancy in response to liraglutide for up to 5 years in the LEADER trial. Diabetes Care 2018;41:620–622
73. Egan AG, Blind E, Dunder K, et al. Pancreatic safety of incretin-based drugs—FDA and EMA assessment. N Engl J Med 2014;370:794–797
74. Drucker DJ. Incretin action in the pancreas: potential promise, possible perils, and pathological pitfalls. Diabetes 2013;62:3316–3323
75. Drucker DJ. Never waste a good crisis: confronting reproducibility in translational research. Cell Metab 2016;24:348–360
76. Chadwick KD, Fletcher AM, Parrula MC, et al. Occurrence of spontaneous pancreatic lesions in normal and diabetic rats: a potential confounding factor in the nonclinical assessment of GLP-1–based therapies. Diabetes 2014;63:1303–1314
77. Azoulay L, Filion KB, Platt RW, et al.; Canadian Network for Observational Drug Effect Studies Investigators. Incretin based drugs and the risk of pancreatic cancer: international multicentre cohort study. BMJ 2016;352:i581
78. Hernández C, Bogdanov P, Corraliza L, et al. Topical administration of GLP-1 receptor agonists prevents retinal neurodegeneration in experimental diabetes. Diabetes 2016;65:172–187
79. Hebsgaard JB, Pyke C, Yildirim E, Knudsen LB, Heegaard S, Kvist PH. Glucagon-like peptide-1 receptor expression in the human eye. Diabetes Obes Metab. 29 April 2018 [Epub ahead of print]. DOI: 10.1111/dom.13339