GLP-1 and the kidney: from physiology to pharmacology and outcomes in diabetes

Marcel H. A. Muskiet[1], Lennart Tonneijck[1]*, Mark M. Smits[1], Michaël J.B. van Baar[1], Mark H. H. Kramer[1], Ewout J. Hoorn[2], Jaap A. Joles[3] and Daniël H. van Raalte[1]*

Abstract | The gastrointestinal tract — the largest endocrine network in human physiology — orchestrates signals from the external environment to maintain neural and hormonal control of homeostasis. Advances in understanding entero-endocrine cell biology in health and disease have important translational relevance. The gut-derived incretin hormone glucagon-like peptide 1 (GLP-1) is secreted upon meal ingestion and controls glucose metabolism by modulating pancreatic islet cell function, food intake and gastrointestinal motility, amongst other effects. The observation that the insulinotropic actions of GLP-1 are reduced in type 2 diabetes mellitus (T2DM) led to the development of incretin-based therapies — GLP-1 receptor agonists and dipeptidyl peptidase 4 (DPP-4) inhibitors — for the treatment of hyperglycaemia in these patients. Considerable interest exists in identifying effects of these drugs beyond glucose-lowering, possibly resulting in improved macrovascular and microvascular outcomes, including in diabetic kidney disease. As GLP-1 has been implicated as a mediator in the putative gut–renal axis (a rapid-acting feed-forward loop that regulates postprandial fluid and electrolyte homeostasis), direct actions on the kidney have been proposed. Here, we review the role of GLP-1 and the actions of associated therapies on glucose metabolism, the gut–renal axis, classical renal risk factors, and renal end points in randomized controlled trials of GLP-1 receptor agonists and DPP-4 inhibitors in patients with T2DM.

[1]Diabetes Center, Department of Internal Medicine, VU University Medical Center, De Boelelaan 1117, 1081 HV Amsterdam, Netherlands.
[2]Department of Internal Medicine, Division of Nephrology and Transplantation, Erasmus Medical Center, s-Gravendijkwal 230, 3015 CE, Rotterdam, Netherlands.
[3]Department of Nephrology and Hypertension, University Medical Centre, Heidelberglaan 100, 3584 CX, Utrecht, Netherlands.
**These authors contributed equally to this work.*
Correspondence to M.H.A.M.
ma.muskiet@vumc.nl

doi:10.1038/nrneph.2017.123
Published online 4 Sep 2017

The International Diabetes Federation estimates that one in 11 adults is living with diagnosed diabetes, predominantly type 2 diabetes mellitus (T2DM)[1]. The prevalence is increasing at such a fast rate that statistical and demographic forecasts have consistently underestimated the trend[2,3]. Patients with diabetes are at increased risk of developing macrovascular and microvascular complications, which cause physical and psychological distress and result in considerable socioeconomic pressure on affected individuals as well as overwhelming costs to global health-care systems.

Fortunately, management of T2DM and prevention of complications has improved substantially over the past 20 years. Control of modifiable risk factors (such as hyperglycaemia, obesity, hypertension, albuminuria, dyslipidaemia and smoking), and widespread use of renin–angiotensin–aldosterone system (RAAS) inhibitors, statins and platelet inhibitors[4], have resulted in a more optimistic outlook for patients[5]. The available data from cohort studies (mostly in high-income countries) suggest that the incidences of T2DM-related myocardial infarction, stroke, amputations and mortality have decreased by >50%[6,7]. The incidence of T2DM-related end-stage renal disease (ESRD) has, however, decreased by only 29%[6] — the lowest rate of decline of all the complications examined. Consequently, a persistently high absolute number of patients with diabetes (20 per 10,000) initiate renal replacement therapy (RRT) every year[7], and diabetes remains the primary cause of chronic kidney disease (CKD) and ESRD, accounting for ~33% of all patients initiating RRT worldwide[8]. Thus, new therapeutic agents or innovative approaches to prevent the onset and progression of diabetic kidney disease (DKD) are urgently needed. Finding new, safe and effective approaches to halt DKD is challenging and a series of therapies and strategies (bardoxolone-methyl, aldose-reductase inhibitors, sulodexide and maximal RAAS inhibition) have failed over the course of drug development, despite encouraging data from preclinical and small clinical studies[9–11].

Key points

- The incretins glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are gut-derived hormones that potentiate insulin secretion and contribute to glucose metabolism through a wide range of physiological actions
- Inhibitors of the incretin-inactivating enzyme dipeptidyl peptidase 4 (DPP-4) and DPP-4-resistant injectable GLP-1 receptor agonists have been developed for the treatment of hyperglycaemia in type 2 diabetes mellitus (T2DM)
- GLP-1 and other gut-derived hormones might directly and/or indirectly regulate electrolyte and fluid homeostasis by influencing feeding and drinking behaviour as well as electrolyte transport in the kidneys and gastrointestinal tract
- GLP-1 receptor (GLP-1R) agonists and DPP-4 inhibitors increase natriuresis in T2DM, possibly through overlapping and distinct mechanisms, and might slightly improve renal haemodynamics in the setting of diabetes-related glomerular hyperfiltration
- Incretin-based therapies seem to directly influence renal physiology and have indirect metabolic and haemodynamic actions that might reduce renal risk in T2DM; considerable interest exists in identifying these glucose-independent renoprotective actions
- Data from clinical trials suggest that GLP-1R agonists and, to a lesser extent, DPP-4 inhibitors marginally improve surrogate renal end points, plausibly beyond the effects of improved glycaemic control

Over the past decade, three new classes of glucose-lowering agents with putative glucose-independent renoprotective potential have been successfully introduced for the treatment of T2DM: glucagon-like peptide 1 receptor (GLP-1R) agonists, dipeptidyl peptidase 4 (DPP-4) inhibitors, and sodium-glucose cotransporter 2 (SGLT2) inhibitors[12,13]. The renoprotective effects and mechanisms of SGLT2 inhibitors in patients with T2DM have been widely discussed[14–16], but advances in understanding the potential benefits of GLP-1R agonists and DPP-4 inhibitors (collectively known as incretin-based therapies) on the renal system have gained less awareness. In 2014, largely based on preclinical evidence and early mechanistic human studies, we suggested that incretin-based therapies may confer renoprotection through multiple mechanisms in T2DM[17]. New data on incretin-based therapy in these patients have accumulated rapidly over the last few years, and substantial progress has been made in understanding the actions of these drugs on the kidney.

In this Review, we discuss the effects of the gut-derived incretin hormone GLP-1 on glucose metabolism and renal physiology and evaluate the direct and indirect pathways by which incretin-based therapies may reduce DKD burden. Finally, we review the effects of GLP-1R agonists and DPP-4 inhibitors on renal outcomes as reported in animal models, pooled registration trials and six landmark cardiovascular outcome studies in T2DM.

The entero-endocrine system

In addition to facilitating absorption and maintaining other functions, the gut is increasingly recognized as the largest endocrine organ in the human body. The gut functions as an early warning system that orchestrates a series of complex physiological responses to changes in the external environment[18]. As such, the entero-endocrine system is believed to facilitate the uptake, distribution and disposal of nutrients, while protecting the integrity of the vulnerable intestinal surface and the whole organism through the enteric innate and adaptive immune system[18–20].

Sixteen major entero-endocrine cell types have been identified to date; these cells are located throughout the gastrointestinal epithelium from stomach to rectum[19]. In response to specific stimuli from the continuously modified luminal content of the intestine (nutrients, fluid, microorganisms and their products, gastrointestinal secretions and pharmaceuticals), entero-endocrine cells release a cocktail of gut hormones from basolateral secretory granules[18–20]. Gut-derived hormones can act locally on other cells (including other entero-endocrine cells) or nerve endings or on organs and tissues at remote sites[18–20] (FIG. 1). Locally, gut hormones seem to be involved in barrier function and in food digestion and absorption by regulating intestinal transit, release of digestive enzymes and induction of nutrient transporters. Suggested peripheral effects include actions on the central nervous system (CNS) to regulate food intake (appetite and satiety); as well as on the pancreas (which contains tissues with endocrine and exocrine roles), liver, skeletal muscle, adipose tissue and vascular system to efficiently absorb and dispose of assimilated nutrients; and on the kidney to adjust urinary excretion of fluid and electrolytes according to intake.

Regulation of glucose metabolism

An oral glucose load elicits a much greater increase in insulin secretion than does intravenous glucose administration (FIG. 2a), owing to insulinotropic signals from the gastrointestinal tract (hormones and/or glucose-responsive nerves)[21,22]. This phenomenon, which is known as the incretin effect, is responsible for up to ~70% of the overall insulin secretory response after nutrient ingestion in individuals with normal oral glucose tolerance[23,24]. Two incretin hormones have been identified in the gut: glucose-dependent insulinotropic polypeptide (GIP) is produced by entero-endocrine K cells, which are predominately localized in the upper gastrointestinal tract[25], whereas GLP-1 is mainly secreted from L cells, which reside throughout the intestine but with increasing abundance towards the distal ileum and colon[26].

GLP-1 is secreted at low tonic rates in the fasting state and the circulating levels of both incretins increase rapidly (within minutes) and transiently upon food intake. K cells and L cells are directly stimulated by luminal glucose via SGLT1, but also secrete incretins in response to other carbohydrates, lipids, proteins, amino acids, bile acids and short-chain fatty acids[27] (FIG. 1). Given the rapid postprandial release of GLP-1 (plasma levels increase from ~5–10 pmol/l when fasting to 15–50 pmol/l within 15–30 mins after eating) and the fairly small number of L cells in the upper gut, an involvement of indirect hormonal (for example, via GIP, cholecystokinin or leptin) or neural loops in relaying the presence of duodenal nutrients to ileal and colonic L cells has been proposed. In addition to acting via neural pathways, incretins exert their insulinotropic activity, at least partly, via interaction with distinct GIP receptors

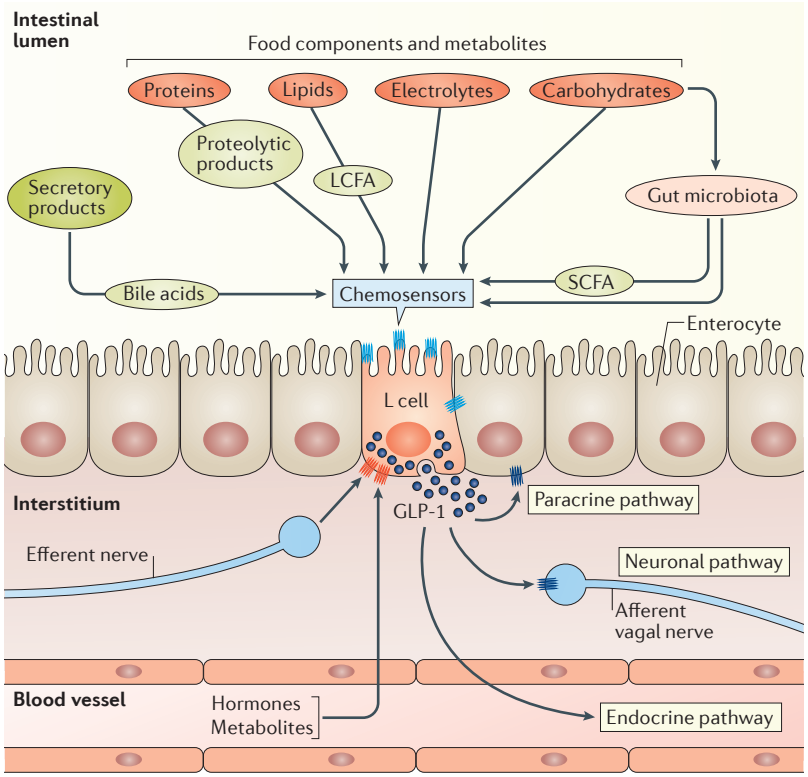

Figure 1 | **The sensory and secretory function of the L cell.** Release of glucagon-like peptide 1 (GLP-1) from L cells is regulated by nutritional, hormonal and neural signals. Food components and metabolites at the luminal side of the L cell are directly sensed by various G protein-coupled receptors that function as chemosensors and trigger exocytosis of GLP-1-containing granules at the basolateral side of the cell. GLP-1 can act through endocrine, paracrine and neuronal pathways to regulate physiological responses in local and/or remote tissues and cell types. These effects are consistent with the widespread and abundant expression of the GLP-1 receptor. LCFA, long-chain fatty acid; SCFA, short-chain fatty acid.

(GIPRs) and GLP-1Rs, which are highly expressed on pancreatic β-cells. In the presence of stimulatory levels of glucose, binding to these G protein-coupled receptors leads to insulin secretion and promotion of insulin transcription and biosynthesis.

A growing body of evidence indicates effects of incretins on pancreatic α-cells and glucoregulatory extrapancreatic tissues[28] (FIG. 3). These findings are not surprising given that GIPRs and GLP-1Rs have been putatively localized in multiple organs and cell types. GLP-1 seems to be a glucose-dependent inhibitor of glucagon release[29], which accounts for ~22%[30] and ~80%[31] of glucagon suppression in the fasting and postprandial states, respectively, mainly through inhibitory factors released from local β-cells (such as insulin) and/or δ-cells (such as somatostatin)[32]. The glucagonostatic effect of GLP-1 might be as important to glucose lowering as its incretin effect. By contrast, under certain circumstances, GIP can stimulate glucagon secretion and thereby antagonize GLP-1 actions[33,34].

GLP-1 also seems to be involved in the central regulation of homeostatic feeding, a process of modifying the rewarding value of food depending on bodily requirements, as derived from signalling of ingested and stored nutrients[35]. GLP-1 dose-dependently enhances satiety signals and reduces appetite resulting in abridged food intake, either directly through GLP-1R stimulation in reward-related brain areas or indirectly via vagal afferents[35–37]. Furthermore, GLP-1 exerts effects on the gastrointestinal tract that facilitate efficient digestion and contribute to energy homeostasis[28,38]. These actions include inhibition of gastric emptying rate and small intestinal peristalsis; exocrine secretion of bile acids, digestive enzymes and bicarbonate; and suppression of endogenous glucose production[28,38]. Finally, GLP-1 seems to contribute to the regulation of nutrient distribution and postprandial energy storage by recruiting the microvasculature to peripheral tissues such as skeletal muscle[28,39] (FIG. 3).

Incretin-based therapies

The incretin effect is severely reduced or lost in patients with T2DM[40–42] (FIG. 2b) and a defective incretin system is a key pathophysiological defect that contributes to glucose intolerance[43]. Meta-analyses that included small, heterogeneous studies showed no systematic differences in the circulating concentrations of GIP[44] and GLP-1 (REF. 45) between individuals with and those without T2DM; however, individual responses to an oral glucose load varied widely, and could be determined by factors such as sex, age and BMI. The most sufficiently powered study to date, which included 1,462 Danish adults, demonstrated that GLP-1 responses to an oral glucose tolerance test are up to 25% lower in individuals with prediabetes or T2DM than in those with normal glucose regulation[46]. Whether a defective incretin system in T2DM is caused by decreased responsiveness of β-cells to GLP-1 and GIP[22] or by hyposecretion of incretin hormones remains unclear. Importantly, the insulinotropic response to exogenous GIP administration is completely lost in T2DM[47], whereas a partially preserved, substantial dose-dependent response to GLP-1 is observed[47,48]. Hence, most pharmacological efforts for treatment of T2DM are directed at amplification of GLP-1-induced glucose lowering in this population.

GLP-1 infusion

Short-term intravenous infusion of GLP-1 at supraphysiological concentrations normalizes or near normalizes fasting plasma glucose (FPG) and postprandial glucose levels (PPG) for up to 7 days in patients with varying severities of T2DM[48–50]. In 2002, a 6-week proof-of-concept study in 19 obese patients with T2DM showed that subcutaneously infused GLP-1 (to achieve plasma levels of ~60–70 pmol/l) reduced the levels of haemoglobin A_{1c} (HbA_{1c}) by ~1.3% and FPG by ~4–6 mmol/l from baseline without inducing hypoglycaemia, improved β-cell function and insulin sensitivity, delayed gastric emptying rate and induced weight loss of 1.9 kg (REF. 51).

GLP-1 has low stability *in vivo* and continuous infusion to overcome this problem has limited clinical applicability for long-term treatment of T2DM. Circulating GLP-1 is rapidly inactivated (<2 min), primarily by the ubiquitous proteolytic enzyme DPP-4

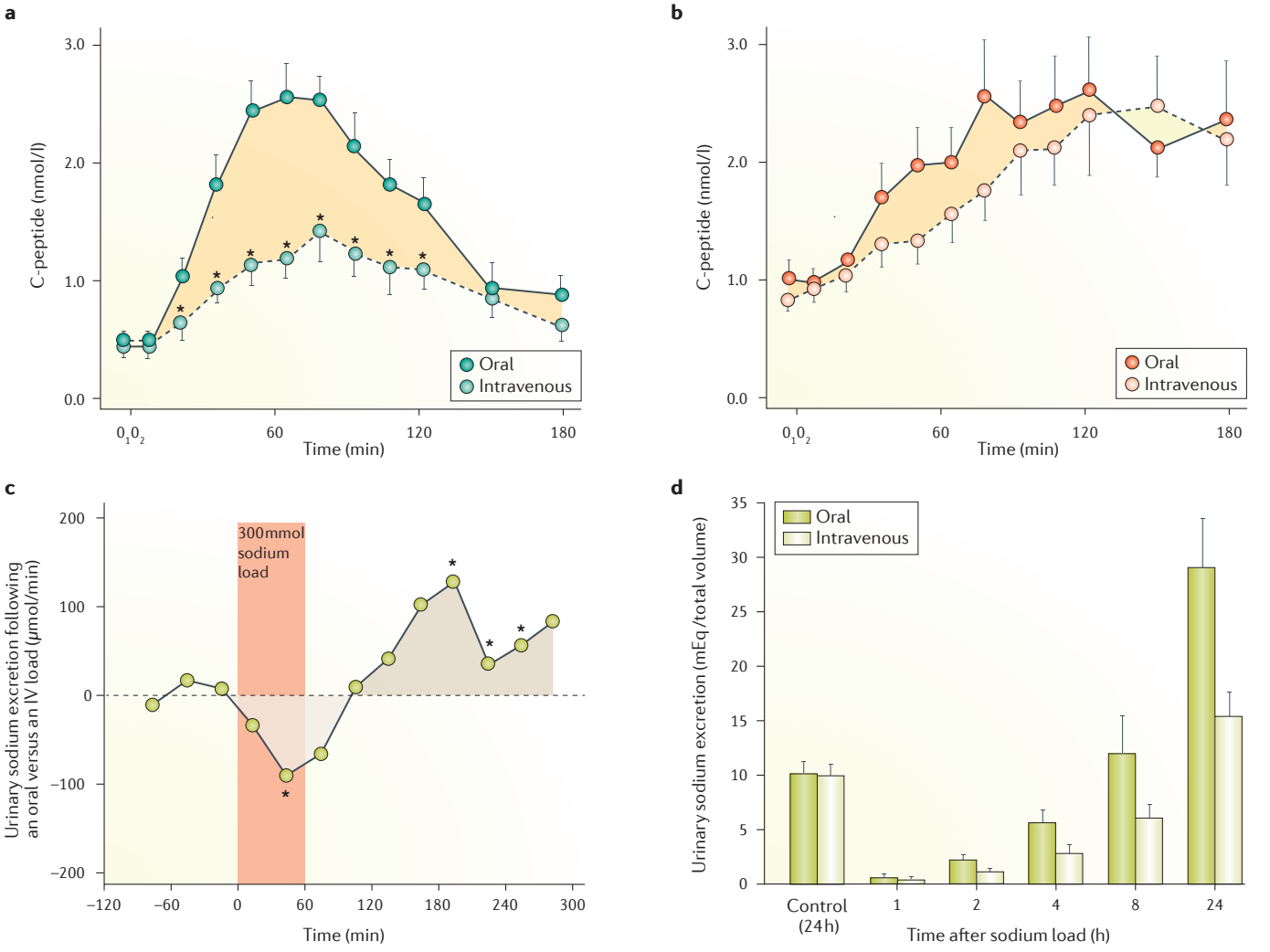

Figure 2 | **Evidence for the incretin effect and the putative gastrointestinal regulation of urinary sodium excretion.** **a,b** | Incremental pancreatic β-cell secretory responses (assessed using C-peptide, which is a marker of endogenous insulin secretion) to an oral glucose load (50 g in 400 ml) or isoglycaemic intravenous (IV) glucose infusion in (part **a**) healthy individuals ($n = 8$) and (part **b**) patients with type 2 diabetes mellitus (T2DM; $n = 14$). In both groups, the isoglycaemic glucose infusion mimicked plasma glucose concentration profiles after glucose ingestion. The incretin effect — defined as the difference in the area under the curve of the C-peptide response to oral versus IV glucose — is, however, markedly reduced or absent in patients with T2DM. **c,d** | An equivalent sodium load is more rapidly excreted by the kidneys when given orally than when given by IV infusion. Relative urinary sodium excretion after an oral compared to IV load of 300 mmol sodium in 2 l volume over 60 min in healthy men ($n = 8$) who were in normal sodium balance with an intake of 150 mmol sodium per day[86] (part **c**). Cumulative urinary sodium excretion during a balanced 10 mEq constant sodium intake 24 h before and following a 100 mEq oral or IV sodium load in healthy men ($n = 8$) (part **d**). Similar to C-peptide, these findings indicate a stimulatory contribution of the gastrointestinal tract to regulation of urinary sodium excretion — the gut–renal axis. *Denotes statistical significance ($P < 0.05$). Parts **a** and **b** reproduced with permission from Springer © Nauck, M. *et al. Diabetologia* **29**, 46–52 (1986). Part **d** reproduced with permission from Wolters Kluwer Health, Inc © Carey, R. M. *Circ. Res.* **43**, 19–23 (1978).

(REFS 52,53) and to a lesser extent by various neutral endopeptidases and aminopeptidases[54–56]. The contribution of the latter may increase in ESRD[57]. DPP-4 is a circulating or membrane-bound serine protease found at numerous sites in the body. This enzyme specifically cleaves dipeptides from the amino terminus of oligopeptides or proteins that contain an alanine (as do incretins) or proline residue at position 2, thereby altering (usually inactivating) their biological activity[17,58]. The truncated metabolites of incretin hormones produced by DPP-4 cleavage do not stimulate insulin secretion. These findings prompted two strategies to extend the *in vivo* half-life of GLP-1 for T2DM therapy and to maintain incretin activity: the use of GLP-1R agonists that are resistant to DPP-4 cleavage, and the use of inhibitors of DPP-4, which prevent proteolytic degradation and inactivation of endogenously secreted incretins.

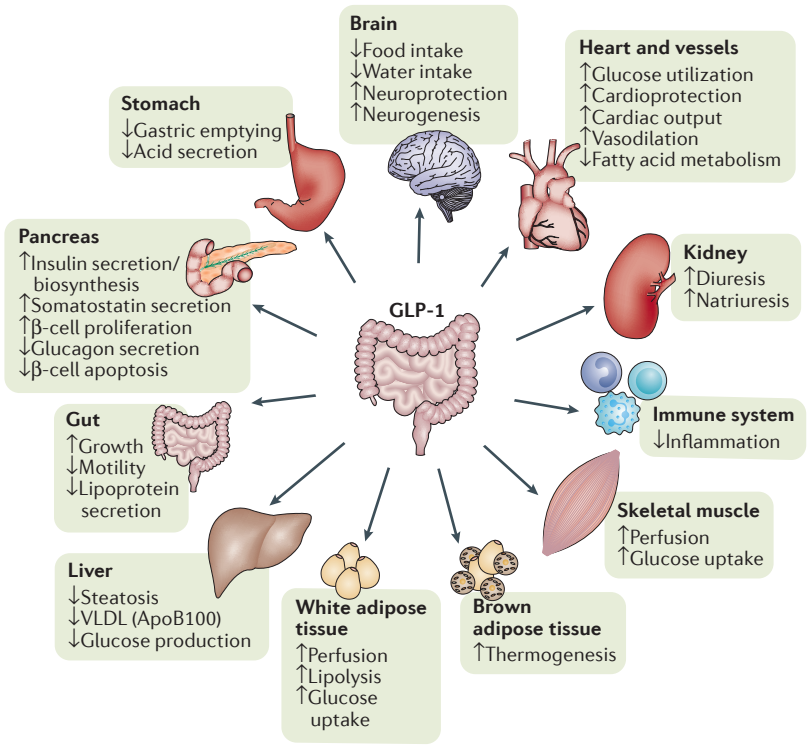

Figure 3 | **Putative actions of glucagon-like peptide 1 (GLP-1).** The best elucidated physiological roles of GLP-1 are those related to pancreatic islet cell function. However, GLP-1 and GLP-1 receptor agonists also have pleiotropic effects on various other tissues and organs, with various potential physiological, pathophysiological and pharmacological implications. VLDL, very low density lipoprotein.

GLP-1 receptor agonists

Several GLP-1R agonist formulations have been introduced for glucose lowering in T2DM (TABLE 1). All of these formulations are administered as a subcutaneous injection and are available for combination therapy with oral antihyperglycaemic agents and basal insulin[13]. These molecules were developed based on human GLP-1 or exendin 4, which is a 39-amino-acid peptide that has 53% homology with GLP-1 and was originally isolated from the saliva of the Gila monster (*Heloderma suspectum*)[59].

In 2005, exenatide (twice-daily) became the first clinically approved GLP-1R agonist for treatment of T2DM[13]. This synthetic version of exendin 4 contains an Ala8Gly substitution that confers resistance to degradation by DPP-4 (REF. 59). Exenatide and the structurally similar GLP-1R agonist lixisenatide largely overcome DPP-4 inactivation *in vivo*, but remain subject to renal elimination and so have a half-life of only ~2–4 h. As such, these compounds are classified as short-acting or prandial GLP-1R agonists with short-lived receptor activation[28,60]. To improve the pharmacokinetics, modified GLP-1 peptides that bind to large carrier molecules (to limit renal clearance) or are co-administered with other chemicals (to delay subcutaneous tissue absorption) have been developed. These long-acting GLP-1R agonists have half-lives of up to a week[28,60]. They include the once-daily GLP-1 analogue liraglutide (which has 97% amino acid sequence identity to human GLP-1) and the once-weekly compounds albiglutide, dulaglutide and a long-acting release formulation of exenatide, which is formulated within biodegradable polymeric microspheres[13]. In addition, semaglutide, which is structurally related to liraglutide, albeit with higher affinity for albumin, is filed for regulatory approval as a once-weekly injection[61].

As pharmacokinetic data and clinical experience with GLP-1R agonists in patients with T2DM and CKD are limited, caution or discontinuation is advised when renal function is severely impaired[62,63] (TABLE 1). The results of a trial of liraglutide in patients with T2DM on chronic dialysis[64], and *post hoc* safety data from a subgroup of 224 patients with T2DM and estimated glomerular filtration rate (eGFR) <30 ml/min/1.73 m^2 enrolled in the LEADER trial of liraglutide[65,66], are eagerly awaited.

Glucose-lowering mechanisms and efficacy. In general, GLP-1R agonists reduce HbA_{1c} levels by ~1.0% compared with placebo[13,28,67]; however, the reductions achieved depend on the choice of agent, dose, baseline HbA_{1c} level and background therapy. Differences between the pharmacokinetic and pharmacodynamic profiles of short-acting and long-acting GLP-1R agonists should be considered when choosing the most appropriate agent for individual patients[28,60].

Short-acting GLP-1R agonists are dosed preprandially, strongly suppress postprandial glucagon levels and substantially retard gastric emptying, which prolongs the rate of glucose entry into the duodenum, blunts absorption of meal-derived glucose, and subsequently diminishes PPG and insulin excursions[28,60]. As plasma drug concentrations decrease rapidly, their effect in the fasting state and on subsequent meals are modest. By contrast, long-acting GLP-1R agonists more strongly reduce FPG owing to uninterrupted glucose-dependent stimulation of insulin secretion (drug concentrations remain elevated throughout the periods between doses)[68,69]. Unlike short-acting GLP-1R agonists, long-acting compounds do not substantially interrupt gastric motility after prolonged administration, resulting in less effect on PPG, as reported in a head-to-head trial comparing liraglutide with lixisenatide[70]. The notable lack of effect on gastric emptying rate is attributed to tachyphylaxis, which is caused by constant GLP-1R activation that induces tolerance to the drug. Further studies should consider which other GLP-1 effects are subject to tachyphylaxis. As such, the ability of liraglutide to inhibit areas in the CNS involved in hedonic feeding after 10 days of treatment are not sustained after 12 weeks[36], and postprandial glucagon suppression was absent at week 12 with liraglutide[71] and was sustained after 3 years of exenatide twice-daily[72].

DPP-4 inhibitors

Five selective and competitive inhibitors of DPP-4 for once-daily oral administration have become available globally since 2006 (TABLE 1). These agents are licensed as monotherapy or as add-on therapy to other antihyperglycaemic drug classes for T2DM treatment[13,73]. DPP-4 inhibitors comprise a diverse group of compounds that can be broadly divided into two groups: agents

Table 1 | **The pharmacokinetic properties and clinical use of incretin-based therapies**[13,73,74,255,256]

Agent	Dose	Approval (year)		Half-life (h)	DPP-4 inhibition (24 h post-dose*)	Elimination	Use in patients with renal insufficiency		
		EMA	FDA				Mild (CrCl 50/60–89 ml/min)	Moderate (CrCl 30–50/60 ml/min)	Severe or ESRD (CrCl <30 ml/min)
Short-acting GLP-1 receptor agonists (subcutaneous injection)									
Exenatide	5–10 µg BID	2005	2005	2.4	NA	Mainly renal	No adjustment	Conservative dose escalation	Not recommended
Lixisenatide	10–20 µg QD	2013	2016	3.0	NA	Mainly renal	No adjustment	No adjustment	Not recommended
Long-acting GLP-1 receptor agonists (subcutaneous injection)									
Exenatide	2 mg QW	2011	2012	NS‡	NA	Mainly renal (~10 weeks to fully clear)	No adjustment	Not recommended	Not recommended
Liraglutide	0.6 mg, 1.2 mg or 1.8 mg QD	2009	2010	11.6–13.0	NA	Peptidases and renal 6%; faeces 5%	No adjustment	No adjustment	Not recommended
Albuglutide	30–50 mg QW	2014	2014	~120.0	NA	Peptidases and renal	No adjustment	No adjustment	Not recommended
Dulaglutide	0.75–1.5 mg QW	2014	2014	~112.8	NA	Peptidases and renal	No adjustment	No adjustment	Not recommended
Semaglutide	0.5–1.0 mg QW	Pending	Pending	165.0–184.0	NA	Peptidases and renal	Unknown	Unknown	Unknown
DPP-4 inhibitors (oral)									
Sitagliptin	100 mg QD	2007	2006	~12.4	>80%	Renal ~87%; faeces ~13%	No adjustment	Dose reduction (50 mg QD)	Dose reduction (25 mg QD)
Vildagliptin	50 mg BID or 50 mg QD plus SU	2007	2007	~2.0	<40% (~80% after 12 h)	Renal ~85%; faeces ~15%	No adjustment	Dose reduction (50 mg QD)	Dose reduction (50 mg QD)
Saxagliptin	5 mg QD	2009	2009	~2.5 (metabolite ~3.1)	~70%	Renal 12–29% (metabolite 21–52%); faeces 22%	No adjustment	Dose reduction (2.5 mg QD)	Dose reduction (2.5 mg QD)
Alogliptin	25 mg QD	2013	2013	~21.0	~75%	Renal ~76%; faeces ~13%	No adjustment	Dose reduction (12.5 mg QD)	Dose reduction (6.25 mg QD)
Linagliptin	5 mg QD	2011	2011	~12.0	>80%	Renal ~5%; faeces ~80%	No adjustment	No adjustment	No adjustment

*Dose may vary in some countries. ‡The pharmacokinetic profile of exenatide QW is similar to that of exenatide BID, except that subcutaneous absorption is prolonged with the QW formulation. BID, twice-daily; CrCl, creatinine clearance; DPP-4, dipeptidyl peptidase 4; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; FAPα, fibroblast activation protein-α; GLP-1, glucagon-like peptide 1; NA, not applicable; NS, not specified; SU, sulfonylurea. QD, once-daily; QW, once-weekly.

that mimic the dipeptide structure of DPP-4 substrates (sitagliptin, vildagiliptin and saxagliptin); and non-peptidomimetic agents (alogliptin and linagliptin)[74]. DPP-4 inhibitors vary in their pharmacokinetic properties and elimination pathways, which determine dosing and might influence clinical usage. Although differences in enzyme selectivity between DPP-4 inhibitors clearly exist in *in vitro* studies[58,74], no evidence exists for pleiotropic effects related to off-target inhibition when the drugs are used therapeutically[58].

The kidneys are important for the final elimination of most DPP-4 inhibitors, which involves glomerular filtration and active tubular secretion through unknown mechanisms. Sitagliptin and alogliptin rely almost exclusively on renal clearance, whereas hepatic metabolism (which generates an active metabolite with ~50% potency) contributes to the elimination of saxagliptin, and hydrolysis adds to the elimination of vildagliptin (~60% of which is achieved by DPP-4 itself). By contrast, the main elimination route for linagliptin is biliary excretion[74]. At a therapeutic dose, linagliptin is mostly protein-bound, which minimizes its renal clearance to <6%; this compound does not, therefore, require dose-adjustment for renal impairment[74]. For the other DPP-4 inhibitors, total exposure to the drug increases proportionally to the degree of GFR decline[63]. Although most of these agents are well tolerated in advanced CKD and even in patients with ESRD (in whom the fraction of the dose removed by haemodialysis is small[75]), specific recommendations for appropriate dose reductions according to CKD severity are in place (TABLE 1).

Glucose-lowering mechanisms and efficacy. The glucose-lowering effect of DPP-4 inhibitors is most notably mediated by preventing the postprandial fall of endogenous GLP-1, thereby enhancing and prolonging insulin secretion and glucagon suppression. As such, DPP-4 inhibitors induce a 1.5–3.0-fold increase in circulating levels of biologically active GLP-1 (REF. 74).This increase is low in comparison to the therapeutic concentrations of GLP-1R agonists, which are equivalent to an ~10-fold increase in endogenous GLP-1 (REF. 76).

In addition to increasing circulating GLP-1 levels, experimental studies in rodents and humans indicate the existence of non-classical glucose-lowering mechanisms of DPP-4 inhibitors[77]. These include inhibition of gut DPP-4 activity, which augments GLP-1-induced activation of autonomic nerves as well as high portal GLP-1 levels that suppress endogenous glucose production; inhibition of pancreatic islet DPP-4 activity, which augments islet cell-produced GLP-1 that directly stimulates insulin and inhibits glucagon secretion; and reduced inactivation of DPP-4 substrates other than GLP-1, which may increase islet cell function and induce additional actions (see below). In contrast to GLP-1R agonists, DPP-4 inhibitors do not delay gastric emptying or increase satiety owing to the relatively low incretin levels that are achieved and/or the antagonizing influences of other DPP-4 substrates. Once DPP-4 is inhibited, glucose lowering plateaus and, consequently, reductions in glucose levels are similar across this drug class without an obvious basis for differentiation regarding efficacy[78]. DPP-4 inhibitors reduce PPG excursions by ~3.0 mmol/l, and FPG levels by 1.0–1.5 mmol/l (REF. 13). In a 2015 meta-analysis of 98 trials of ≥12 weeks duration, DPP-4 inhibitors as monotherapy or as add-on therapy to other oral agents reduced HbA_{1c} levels by 0.77% (95% CI 0.82–0.72%) from a mean baseline of 8.05%[79].

Other incretin-independent effects. DPP-4 is a widely and abundantly expressed multifunctional enzyme that transduces numerous actions as a transmembrane and soluble circulating molecule. The challenge of understanding DPP-4 action is amplified by the fact that, in addition to GLP-1 and GIP, DPP-4 cleaves many bioactive peptides, including chemokines, neuropeptides and regulatory peptides[17,58]. Cleavage by DPP-4 can inactivate peptides and/or generate new bioactive moieties, which might impact numerous cell types and organs, including the kidney, theoretically resulting in numerous pleiotropic benefits and risks[17,58]. Despite a host of preclinical studies that have identified and characterized putative DPP-4 substrates *ex vivo* and in experimental models[58], the understanding of key non-incretin substrates with clinically relevant actions remains limited.

The gut–renal axis

Harnessing the pharmacological properties of gut hormones has sparked interest in the properties and therapeutic potential of entero-endocrine cells in various medical disciplines, including nephrology, as well as in defining other regulatory actions of GLP-1 (and consequently incretin-based therapies) beyond glycaemia.

Entero-endocrine cells seem to contribute to the physiological control of water and electrolyte balance upon meal ingestion by affecting the CNS to adjust thirst and, to a lesser extent mechanisms that affect solute intake; intestinal transport to control fluid and electrolyte absorption and secretion; intracellular and extracellular compartments to dispose the absorbed content; and the kidney to stimulate excretion or reabsorption of fluid and electrolytes. This system is very flexible. For example, consumption of a potassium-rich meal, which often contains more potassium than the total extracellular potassium content, would be potentially lethal if absorbed potassium were to remain in the extracellular fluid[80,81]. Intracellular redistribution and urinary excretion rapidly removes excessive potassium, particularly in the replete state. As renal electrolyte homeostasis is slowly regulated by circadian rhythms and numerous circulating hormones, a putative rapid-acting gut–renal axis might assist renal solute excretion in response to acute solute ingestion[17,81–83], forming a crucial feed-forward loop.

Effects on tubular transport

Seminal studies that support the concept of the gut–renal axis used similar methodologies to experiments that led to discovery of the incretin effect. For example, depending on sodium balance, an equivalent sodium load is more rapidly excreted by the kidneys when given orally than when administered intravenously in many, but not all, animal and human studies[82,84–87] (FIG. 2c,d). This result seems to be independent of changes in the levels of circulating atrial natriuretic peptide (ANP) and aldosterone[82]. In addition to gut-assisted sodium homeostasis, several lines of evidence suggest similar feed-forward loops for potassium[81–83] and phosphate balance[82,83], and perhaps other electrolytes[82]. The gut has been suggested to directly detect changes in the levels of ingested electrolytes (FIG. 1) and couple these changes to release of hormones and/or activation of neural pathways that regulate renal tubular and gastrointestinal transport. Several gut hormones and peptides have been proposed to be effectors of, or have a role in, the gut–renal (natriuretic) axis, including gastrin (via interaction with renal dopamine[88]), ghrelin, uroguanylin, guanylin, secretin, vasoactive intestinal polypeptide, peptide YY (PYY) and GLP-1 (Supplementary information S1 (table))[82,87].

From a pathophysiological perspective, an impaired gut–renal axis in urinary sodium excretion might contribute to salt-sensitive hypertension. Whether this impairment would involve hyposecretion or reduced receptor signalling of entero-endocrine-cell-derived hormones, or conversely, inability of these signals to suppress antinatriuretic systems (such as the RAAS) in response to a salt load, is unclear. Unravelling the gut–renal axis concept, the mediators and their potential pathophysiological contribution to salt-sensitive hypertension might be important for the development of novel targeted therapies.

Effects on renal haemodynamics

Although gut hormones can vary tubular electrolyte handling without any substantial fluctuation in the filtered load, direct or indirect signals from the gastrointestinal

tract on postprandial renal haemodynamics can also facilitate renal solute excretion. For example, after ingestion of a high-protein meal, a physiological increase in renal blood flow increases GFR independent of changes in arterial pressure, which enhances the filtered load of circulating solutes[89,90]. This postprandial GFR increase might have an important function, as protein catabolism produces nitrogen waste products (urea, uric acid, ammonia and creatinine) and other metabolites (including phosphates, sulphates and protons) that require renal excretion. Postprandial hyperfiltration might thus be a useful mechanism to rapidly excrete

Box 1 | **Putative renal distribution of GLP-1 and membrane-bound DPP-4**

GLP-1

The majority of glucagon-like peptide 1 (GLP-1) actions *in vivo* are transduced by the GLP-1 receptor (GLP-1R). This G-protein-coupled receptor was originally identified in islet β-cells, but its expression has now been reported in numerous extrapancreatic tissues, including the lung, brain, enteric and peripheral nervous systems, lymphocytes, heart and blood vessels and various locations in the kidney. Most of the commercially available antisera that are used to detect GLP-1R expression (by immunohistochemistry or western blot analysis) are neither sensitive nor specific, and important control experiments are frequently absent. Furthermore, interspecies differences might hamper exact mapping of the distribution of a functional GLP-1R. A study that used the most extensively validated monoclonal antibody to date showed that in primate kidneys, GLP-1R is exclusively expressed in preglomerular vascular smooth muscle cells and juxtaglomerular cells[106].

Membrane-bound DPP-4

Dipeptidyl peptidase 4 (DPP-4) is highly active and abundantly expressed in the kidney. The highest level of expression of DPP-4 is at the brush border of the S1–S3 segment of the proximal tubule (where it is functionally coupled to intestinal sodium–hydrogen exchanger isoform 3). Lower expression levels are present at other sites in the nephron and tubulointerstitium. The results of studies that analysed the renal distribution of GLP-1R and/or DPP-4 in the kidneys of various organisms are summarized below.

Location	Species	mRNA or protein	Detection method	Refs
GLP-1R				
Preglomerular* vascular smooth muscle cells	Monkey; human	Protein	Immunohistochemistry	106
	Rat	Protein	Autoradiography of ^{125}I-labelled GLP-1, exendin 4 (GLP-1 agonist) and exendin 9–39 (GLP-1R antagonist)	103
Hilar and intralobular arteries	Human	Protein	Autoradiography of ^{125}I-labelled GLP-1	105
Glomerular capillary and vascular walls	Mouse	mRNA	*In situ* hybridization; RT-PCR	102
Glomerular endothelial cells and macrophages	Rat	Protein	Immunofluorescence	104
Glomerulus (not specified)	Rat	mRNA	RT-PCR	101
Juxtaglomerular cells	Monkey; human	Protein	Immunohistochemistry	106
	Rat	Protein	Immunohistochemistry	103
Proximal tubule	Rat	mRNA	RT-PCR	101
	Pig; human	mRNA, protein	RT-PCR, immunocytochemistry, immunohistochemistry, Western blotting	107
	Rat	Protein	Autoradiography of ^{125}I-labelled GLP-1, exendin 4 (GLP-1R agonist) and exendin 9–39 (GLP-1R antagonist)	103
Membrane-bound DPP-4				
Preglomerular vascular smooth muscle cells	Rat	mRNA, protein	RT-PCR, Western blotting	252
Mesangial cells	Rat	mRNA, protein	RT-PCR, Western blotting	252
Podocytes	Rat	Protein	Immunohistochemistry	253
Proximal tubule	Pig; human	mRNA	RT-PCR, immunocytochemistry	107
	Rat	Protein	Immunohistochemistry	253
Loop of Henle, distal convoluted tubule, connecting tubule, cortical collecting duct	Rat	mRNAs	Deep sequencing of RNA species	254

*Afferent arterioles, interlobular arteries and arcuate arteries.

excess or potentially injurious gut-absorbed solutes and catabolic wastes. Numerous gut-derived hormones have been reported to influence renal haemodynamics (Supplementary information S1 (table)). If total nephron capacity is already maximally used in the fasting state (that is, glomerular hyperfiltration), as regularly seen in patients with T2DM and/or advanced CKD to compensate for loss of renal function[90], the contribution of gut-mediated renal haemodynamic changes to acute solute excretion in the postprandial state may be minimal.

Role of GLP-1

An acute oral NaCl load in rats[91,92] and an acute water load in humans[93] have both been reported to increase total circulating GLP-1 levels within 5 min. However, these experiments had important limitations (such as limited GLP-1 measurements and inadequate control experiments) and other studies in rats[94] and humans[95] have as yet not confirmed these observations. How L cells (or other GLP-1-producing cells, for example in the hindbrain) could be stimulated by an oral sodium or water load is unknown. Although sodium arising at the luminal side of the L cell may directly trigger GLP-1 secretion (for example, through glucose-coupling via SGLT1 or Na^+-dependent amino acid transporters[27]), indirect neural or hormonal stimuli might be more likely given the rapidity of the response (FIG. 1).

In line with the role of GLP-1 in the central regulation of feeding[35], peripheral and central GLP-1R agonist administration reduces water and saline intake[94,96–100], which seems to be independent of food intake[99]. Conversely, rats injected with the GLP-1R blocker exendin 9 drank more fluid than vehicle-treated rats in dipsogenic and nondipsogenic conditions, suggesting that endogenous GLP-1 tone suppresses drinking behaviour. This effect might involve GLP-1-producing cells in the nucleus solitarius[94]. In healthy males, GLP-1 infusion decreased *ad libitum* water intake after a salty breakfast by 36%, without affecting serum sodium concentration[98]. In addition to directly affecting drinking behaviour, GLP-1 has been suggested to reduce the need for water consumption by decreasing sodium uptake by the gut, possibly by inhibiting intestinal sodium–hydrogen exchanger isoform 3 (NHE3) activity[98]. In line with this hypothesis, three of the nine volunteers in the study dropped out during GLP-1 infusion owing to osmotic diarrhoea[98].

GLP-1R is expressed in the kidney, with studies of various quality reporting the presence of a functional receptor in proximal tubular cells and in the renal vasculature[101–107] (BOX 1). Although a study that used the most extensively validated monoclonal antibody for GLP-1R to date showed that GLP-1R was exclusively expressed in the preglomerular vascular smooth muscle cells and juxtaglomerular cells of primate kidneys[106], uncertainties remain regarding receptor localization. DPP-4 is highly active as a transmembrane molecule in several renal cell types, particularly podocytes and proximal tubular cells[108]. Proposed direct effects of GLP-1 (via its receptor) and associated therapies on renal physiology (tubular and renal haemodynamic actions) are discussed below. GLP-1 might also indirectly affect kidney function, for example through interactions with other hormones.

Renal effects of incretin-based therapies

Tubular effects

Involvement of GLP-1 in the gut–renal axis was first suggested in 1996 when exogenous GLP-1 was shown to dose-dependently increase natriuresis and diuresis in rats[97]. GLP-1-induced renal sodium excretion and increased urine flow was confirmed in short-term infusion studies involving nondiabetic and diabetic animals[17], healthy volunteers[109], insulin-resistant obese males and patients with T2DM[110]. Furthermore, GLP-1R-blockade with exendin 9 in fasting rats reduced urinary sodium excretion and urinary flow, suggesting a tonic modulatory role of GLP-1 on renal sodium handling[111].

With regard to GLP-1R agonists, acute intravenous infusion of exenatide in healthy males[112] and patients with T2DM[113], and a single subcutaneous injection of liraglutide in patients with T2DM[114], increased both absolute and fractional sodium excretion. Most studies to date are 3–72 h in duration, and only limited data on long-term sustainability of this natriuretic effect are available. In hypertensive patients with T2DM, liraglutide-induced increases in 24 h and night-time urinary sodium excretion were reported to be sustained after 3 weeks of therapy[115], but the results of our placebo-controlled liraglutide study in insulin-naive patients with T2DM suggest that this response disappears before week 12 (REF. 116). This loss of effect is compatible with the GLP-1R desensitization that has been observed for other regulatory systems after prolonged treatment with a long-acting GPL1R agonist. Alternatively, it might indicate compensatory activation of renal sodium transporters or reduced dietary sodium intake. Interestingly, data from our group suggest a contrasting sustained natriuretic effect of the short-acting GLP-1R agonist lixisenatide in glargine-treated patients with T2DM for up to 8 weeks of treatment, which might reflect preserved efficacy caused by intermittent GLP-1R stimulation[117]. DPP-4 inhibitors also promote natriuresis, as demonstrated in animals[101,118,119] and in patients with T2DM after 2–4 weeks of sitagliptin treatment (this effect disappeared before week 12)[116,120].

GLP-1R-mediated natriuresis and diuresis seem to involve inhibition of NHE3, which is located at the brush border of the renal proximal tubule bound to a complex that also contains DPP-4 (REFS 121,122). Pharmacological doses of GLP-1 or GLP-1R agonists increase intrarenal cAMP generation, protein kinase A (PKA) activation and phosphorylation of NHE3 at the PKA consensus sites Ser552 and Ser605 (REFS 101,111,123),which reduces its activity. Conversely, GLP-1R blockade with exendin 9 reduces renal cortical phosphorylation at Ser552 of NHE3 (REF. 111). In animals and humans, acute GLP-1R agonist infusion increases renal lithium clearance (a marker of proximal tubular sodium reabsorption)[101,109,114,124] and urinary pH[110,112,113], supporting the notion that NHE3 is indeed involved. As acute GLP-1R agonist administration increased mean arterial pressure by ~5 mmHg in most reported

human studies[113,114], pressure natriuresis (characterized by a rapid coordinated decreases in both NHE3 surface distribution and Na^+/K^+ ATPase activity[125]) might be at least partly involved in the reported acute natriuresis. A similar mechanism could explain the natriuretic effects of 8-week lixisenatide treatment[117]. DPP-4 inhibitors might also inhibit NHE3 activity, albeit through a tyrosine kinase signalling pathway rather than via PKA activation[122]. Furthermore, DPP-4 inhibitors might redistribute NHE3 (along with a small fraction of DPP-4)[121], and stimulate NHE3-independent sodium excretion[17,126] (see below).

Other factors might also be involved in the natriuretic properties of both incretin-based drug classes. Several factors with a role in glucose metabolism, including insulin, ATP and glucose itself, regulate NHE3 and SGLTs in the kidney, suggesting indirect natriuretic actions of GLP-1. Moreover, interaction of GLP-1R agonists with natriuretic and antinatriuretic factors, and contributions of other DPP-4 substrates in the case of DPP-4 inhibitors, have been suggested to mediate increased sodium excretion[17].

First, GLP-1R agonists might reduce circulating levels and post-receptor actions of components of the RAAS[87,127]. GLP-1 infusion in healthy young males[109], and a single-dose of liraglutide in patients with T2DM[114], decreased plasma levels of angiotensin II by ~20%. Similarly, GLP-1 infusion reduced plasma renin activity in healthy males[128]. However, we and others were unable to confirm effects of acute or prolonged GLP-1R agonist treatment[113,115–117,128,129] or DPP-4 inhibitor therapy[116,120] on circulating RAAS components. Second, an indirect role for neural pathways might be involved, as intra-cerebroventricular GLP-1 administration also rapidly stimulates urinary excretion of water and sodium[97]. Third, a stimulating effect on natriuretic peptides should be considered. Although a gut–cardiac GLP-1R–ANP axis was identified in nondiabetic rodents[130], subsequent studies of the effects of GLP-1 infusion in healthy males[128,131] and of short-term liraglutide therapy in patients with T2DM[114,115], did not show increases in circulating pro-ANP or ANP levels that could explain the urinary sodium excretion. Finally, studies in mice lacking a functional GLP-1R have demonstrated natriuresis following DPP-4 inhibitor, but not GLP-1R agonist, administration, suggesting that the natriuretic effects of DPP-4 inhibitors are in part GLP-1-independent[119,126]. Elevated levels of intact stromal cell-derived factor 1α (SDF1α) might mediate this DPP-4-inhibitor-induced natriuretic response, likely via the Na^+–Cl^- cotransporter or the epithelial sodium channel in the distal convoluted tubule[119].

A placebo-controlled RCT in 32 patients with T2DM showed that 1 month of sitagliptin treatment augmented fractional sodium, but not lithium, excretion, suggesting increased distal tubular natriuresis; this effect was paralleled by an increase in intact plasma SDF1α levels and a decrease in truncated SDF1α levels[120]. However, other DPP-4 substrates have also been associated with RAAS interaction and renal sodium excretion, including neuropeptide Y (NPY), PYY, substance P and brain natriuretic peptide[17]. These substrates might contribute to the GLP-1-independent tubular effects of DPP-4 inhibitors[17].

In addition to natriuresis and diuresis, GLP-1R agonists, and to lesser extent DPP-4 inhibitors, have been reported to increase free water clearance in hyperhydrated rats[132,133] and humans[134], as well as absolute and fractional excretion of potassium[101,113,118,124], chloride[92,110,118,126] and calcium[109,110] in the acute setting (Supplementary information S2 (table)). Which nephron segments and transport proteins are involved, and whether these effects are secondary to the natriuretic and alkalizing effects of GLP-1R agonists and DPP-4 inhibitors, is unknown. In addition, the clinical relevance of these changes in tubular electrolyte handling requires further research.

Haemodynamic effects

The renal haemodynamic actions of GLP-1 seem to be independent of its natriuretic effect in individuals without diabetes. As expected, GLP-1R blockade with exendin 9 increased NHE3 activity and reduced natriuresis in rats, but this effect was paralleled by a paradoxical decrease in GFR (increased proximal tubular reabsorption should inhibit tubuloglomerular feedback signals, leading to reduced afferent arteriolar resistance and increased GFR)[111].

The physiological role of GLP-1-induced glomerular hyperfiltration might be to enhance the filtered electrolyte load after meal ingestion[126]. Single-nephron GFR was acutely raised by up to 50% in rats without diabetes after GLP-1 infusion[103,118,124] or administration of exenatide[135] or liraglutide[118]. Similar, albeit smaller, GFR increases in nondiabetic rodents have been observed with DPP-4 inhibitors[101,111,118]. Acute GLP-1 infusion did not affect GFR or effective renal plasma flow (ERPF) in healthy men[109,128], but exenatide infusion increased GFR, ERPF and estimated glomerular hydraulic pressure (P_{GLO}) in overweight but otherwise healthy males[112]. A direct vasodilatory effect on the afferent arteriole (through GLP-1R-dependent actions on vascular smooth muscle cells) may be involved[103,112]. In rodents, GLP-1R blockade with exendin 9 attenuated GLP-1-induced increases in renal blood flow and reductions in autoregulatory responses to increased pressure in afferent renal arterioles of isolated murine kidneys[103]. In addition, in healthy males, exenatide infusion acutely reduced estimated afferent renal arteriolar resistance, which seems to be, at least in part, dependent on nitric oxide (NO) availability[112]. These findings imply that GLP-1R agonists are proximal diuretics and renal vasodilators that under healthy conditions only mildly influence tubuloglomerular feedback[135,136].

Interestingly, the stimulatory effect of exendin 4 and DPP-4 inhibitors on GFR and renal blood flow were absent in a preclinical model of diabetes[126], and clinical studies suggest that incretin-based therapies reduce GFR and may ameliorate glomerular hyperfiltration[110,137–139]. The integrated GFR response seems to be determined by the magnitude of the direct vasodilator effect of GLP-1R activation in the afferent arteriole versus the activity of factors associated with glomerular hyperfiltration in diabetes[90] that are allegedly inhibited by GLP-1R agonists (FIG. 4). In the setting of insulin resistance or diabetes, the magnitude of the

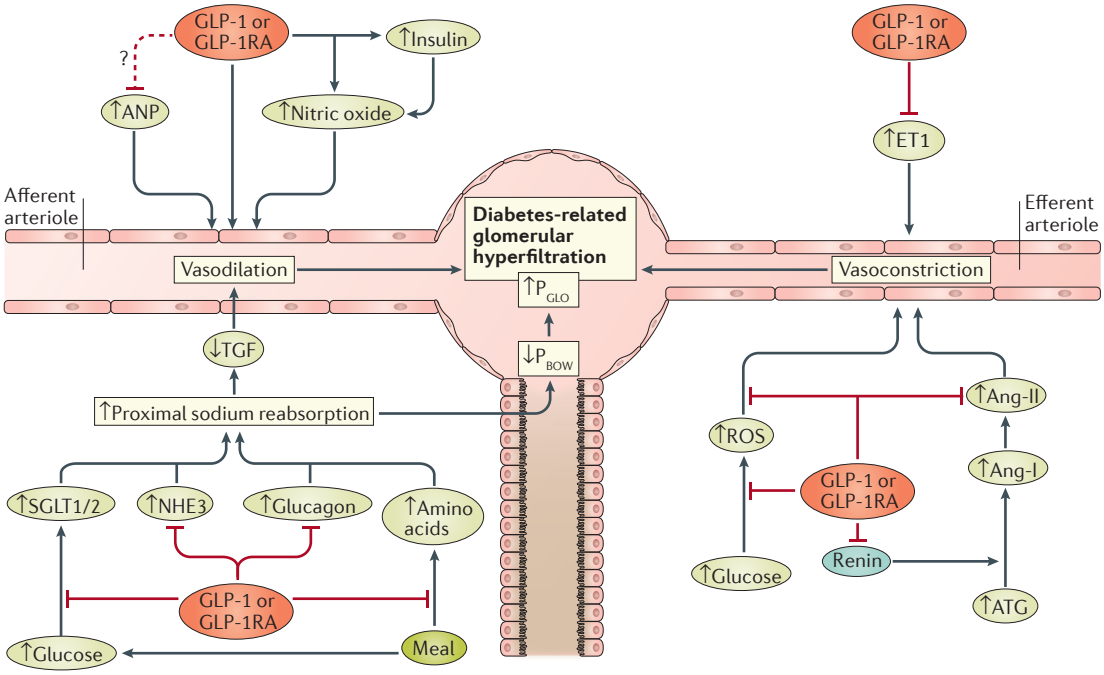

Figure 4 | **Effects of glucagon-like peptide 1 (GLP-1) and GLP-1 receptor agonists (GLP-1RAs) on renal haemodynamics in diabetes mellitus.** Several vascular and tubular factors are implicated in fasting and postprandial glomerular hyperfiltration in the setting of diabetes. These factors result in a net reduction in afferent renal arteriolar resistance, a net increase in efferent renal arteriolar resistance and/or a reduction in hydraulic pressure in Bowman space (P_{BOW}), and thereby an increase in glomerular hydraulic pressure (P_{GLO}) and single nephron glomerular filtration rate. GLP-1RAs are associated with direct GLP-1R-mediated and, at least in part, nitric oxide-dependent vasodilation of the afferent renal arteriole, as well as indirect inhibition of vascular and tubular factors that are putative mediators of glomerular hyperfiltration in diabetes. The integrated effect of incretin-based therapy on renal haemodynamics seems to be the result of direct vasodilative actions and inhibition of pathways of glomerular hyperfiltration. Theoretically, this effect is dependent on baseline phenotypic characteristics and co-medication. Ang-I, angiotensin I; Ang-II, angiotensin II; ANP, atrial natriuretic peptide; ATG, angiotensinogen; ET1, endothelin 1; NHE3, sodium–hydrogen exchanger isoform 3; ROS, reactive oxygen species; SGLT, sodium–glucose co-transporter; TGF, tubuloglomerular feedback.

direct vasodilator effect of GLP-1R might be reduced owing to impaired NO-dependent vasodilation, whereas the inhibitory role of GLP-1 on factors associated with glomerular hyperfiltration could be substantial. Collectively, these effects might result in a net neutral effect or reduction in GFR upon administration of GLP-1 or incretin-based therapy in this population. Thus, glomerular hyperfiltration (a putative renal risk factor[90]) might be an essential milieu for incretin-based therapies to confer renoprotective alterations in renal haemodynamics[140]. This hypothesis is consistent with the divergent renal actions of the SGLT2 inhibitor empagliflozin that are seen in hyperfiltering versus non-hyperfiltering patients with T1DM[141].

Indeed, in early studies in diabetic rodents, 4–8-weeks of administration of exendin 4 (REFS 104,142), liraglutide[102] or linagliptin[119] significantly reduced glomerular hyperfiltration. In line with this finding, infusion of GLP-1 decreased creatinine clearance from 151 ml/min to 142 ml/min in 16 obese insulin-resistant males (four of whom had T2DM), which was in contrast to neutral effects observed in healthy subjects[110]. In an uncontrolled study involving 31 patients with T2DM, liraglutide was associated with an acute reduction in eGFR (paralleled by albuminuria) and subsequent stabilization up to 49-days of therapy, which was reversible after 3-week washout of the drug, suggesting a renal haemodynamic effect[138]. Re-initiation of liraglutide therapy in a 1-year extension of the trial showed a similar eGFR pattern[137]. However, subsequent studies do not support the notion that incretin-based drugs improve renal haemodynamic function. Although an initial drop in eGFR was observed after 2 weeks and 6 weeks of liraglutide or sitagliptin treatment[116], reminiscent of the effects of angiotensin-converting enzyme inhibitors and SGLT2 inhibitors[90], the eGFR trajectory did not differ from placebo. In RCTs of 12–30 weeks duration, GLP-1R agonist or DPP-4 inhibitor treatment did not influence initial changes in or the slope of eGFR in patients with T2DM with[143–146] or without[145,147–149] renal impairment. Moreover, acute administration of GLP-1 (REF. 129), exenatide[113] or liraglutide[114], or 12 weeks of liraglutide or sitagliptin treatment[116] did not affect fasting renal haemodynamics or estimated P_{GLO} in patients with T2DM and presumed late-phase hyperfiltration (that is, filtration fraction of ~25% in the setting of GFR >60 ml/min/1.73 m^2). Also, 12 weeks of liraglutide versus placebo in albuminuric patients with T2DM did not significantly affect GFR, although GFR decreased by >30% in two patients with early

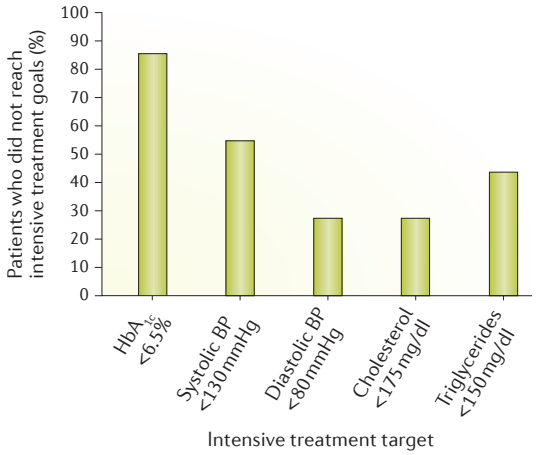

Figure 5 | **Risk factor control in the intensive treatment group of the Steno-2 trial in patients with type 2 diabetes mellitus and microalbuminuria[151].** Percentage of patients in the intensive therapy group ($n = 80$) who did not reach the intensive treatment goals at a mean follow-up of 7.8 years. To convert values for cholesterol to mmol/l, multiply by 0.02586. To convert values for triglycerides to mmol/l, multiply by 0.01129. BP, blood pressure; HbA_{1c}, haemoglobin A_{1c}.

phase hyperfiltration (GFR >135 ml/min/1.73 m^2)[139]. Finally, 4 weeks of linagliptin therapy versus placebo[150] or 8 weeks of lixisenatide therapy versus insulin glulisine[117] in patients with T2DM did not affect fasting or postprandial renal haemodynamics, respectively. In conclusion, GLP-1R agonists seem to induce glomerular hyperfiltration under physiological conditions by reducing afferent arteriolar resistance, but maintain or might slightly improve renal haemodynamic function in patients with T2DM, likely depending on the individual baseline phenotypic characteristics (FIG. 4).

Incretin-based therapies and renal risk factors

The impact of timely, intensive, multifactorial treatment on renal outcome in high-risk patients with T2DM was clearly shown in the Steno-2 trial, in which this strategy lowered the risk of nephropathy by 61% after a mean follow-up of 7.8 years, and slowed renal function loss resulting in a reduced risk of ESRD after 21 years[151,152]. In this trial, which included 160 participants, and in clinical practice, however, residual renal risk remains high[4,151]. Some of this excess risk might be reduced by narrowing the gap between recommended and established risk factor control. In Steno-2, a considerable proportion of the participants in the intensive treatment group did not achieve the set targets[151] (FIG. 5). Similarly, from 1999 to 2010, almost half of US adults with diabetes did not meet recommended goals for diabetes care[153]. Of note, all antihyperglycaemic drug classes have pleiotropic effects that could favourably or unfavourably influence renal risk profile and, as such, aid or oppose the treat-to-target strategy[4]. Below, we discuss off-target effects of incretin-based therapies on important renal risk factors, the clinical relevance of which is reviewed elsewhere[4].

Body weight and composition

Treatment with GLP-1R agonists is associated with reductions in body weight and waist circumference, albeit with much variation in individual responses and within class differences[13]. By contrast, DPP-4 inhibitors do not affect body weight[154]. In a 2015 meta-analysis[155] of 51 RCTs (mean duration 31 weeks), GLP-1R agonists were shown to induce weight loss of 0.79–1.38 kg compared with placebo, and 1.00–7.30 kg compared with antihyperglycaemic drug classes that are associated with weight gain (insulin, sulfonylurea and thiazolidinediones)[154]. Of note, liraglutide-induced weight loss is dose-dependent up to 3.0 mg once daily. A study that included 3,731 non-diabetic obese individuals (BMI ≥30 or ≥27 in those with dyslipidaemia or hypertension), showed that 56 weeks of treatment with liraglutide 3.0 mg resulted in a mean weight loss of 5.8 kg (~5%) compared with placebo[156]. This compound has now been approved for weight management in adults with BMI ≥30 or ≥27 in those with weight-related complications such as dysglycaemia (pre-diabetes or T2DM), hypertension, dyslipidaemia or obstructive sleep apnoea[157]. GLP-1R-agonist-induced reductions in body weight are associated with reductions in total body fat, particularly trunk or visceral fat, rather than in lean tissue mass[158,159]. This effect seems to be the result of a GLP-1R-mediated reduction in appetite and food intake, rather than of increased energy expenditure[35].

Several DPP-4 substrates are pharmacological regulators of food intake (for example PYY, NPY and GLP2), but unlike GLP-1R agonists, DPP-4 inhibitors do not supress appetite or induce satiety. The weight neutral effects of DPP-4 inhibitors might reflect the only modest elevation in intact GLP-1 levels (insufficient to control satiety) that they induce and/or an unfavourable balance between levels and activity of anorectic and orexigenic peptides (TABLE 2).

Blood pressure

Acute administration of GLP-1R agonists increases heart rate and leads to a transient increase in blood pressure in normotensive and hypertensive individuals[28,113,114,128,160,161]. Sustained treatment with both incretin-based drug classes in patients with T2DM is, however, associated with a persistent increase in heart rate but a clinically relevant drop in blood pressure, which is not strictly dependent on weight loss[162,163]. In a meta-analysis of 60 RCTs, systolic blood pressure was significantly reduced with liraglutide (−3.59 mmHg) and albiglutide (−3.67 mmHg), and non-significantly reduced with exenatide (−2.60 mmHg) and dulaglutide (−1.62 mmHg) compared with placebo[162]. The blood-pressure-lowering effect of GLP-1R agonists does not seem to be dose-dependent[164].

Although blood-pressure-lowering effects of DPP-4 inhibitors in individual trials are modest, a 2016 meta-analysis of 15 studies showed mean reductions in systolic and diastolic blood pressure of 3.04 mmHg and 1.47 mmHg, respectively, compared with placebo or no treatment[163]. Mechanisms linking sustained GLP-1R signalling to blood pressure reductions are

Table 2 | **Glucose-independent effects of incretin-based therapies on renal risk factors in type 2 diabetes mellitus**

Renal risk factor	GLP-1RA	DPP-4 inhibitor	Putative GLP-1-mediated mechanisms	Putative GLP-1-independent mechanisms of DPP-4 inhibitors
Obesity	Decrease	Neutral effect	↓Appetite (direct effect on CNS or via vagal afferents, ↓GEE* and ↑nausea) ↑Energy expenditure[35]? ↑Natriuresis and/or diuresis?	Effect possibly counteracted by ↑PYY (1–36) and ↓PYY (3–36)[257,258‡]
Blood pressure	Decrease	Decrease or neutral effect	↓Body weight ↑Endothelial independent vasodilation[259,260]? ↑Natriuresis[261§] ↓Intestinal sodium reabsorption[98]? ↓Sodium intake (direct effect on CNS)? ↓RAAS activity[87,127]? ↑ANP[130]?	↑Natriuresis (↑SDF1α[119], ↓DPP-4/NHE3 complex[262]?, ↑BNP[263]) ↑Vasodilation (↑BNP[263], ↑bradykinin) Effects possibly counteracted by ↑substance P (↑SNS activity) and ↑NPY (potentiates SNS activity) during concomitant ACE inhibition[127‖]
Dyslipidaemia	Decrease	Neutral effect	↓Body weight ↓Intestinal lipid uptake (partly by ↓GEE*) ↓Hepatic lipoprotein synthesis and secretion ↑Insulin sensitivity (partly by ↓body weight) ↑Insulin and ↓glucagon ↑Triglyceride uptake in white adipose tissue ↑Brown adipose tissue activation[169]	Effects possibly counteracted by factors related to steroid metabolism[264]
Inflammation and fibrosis	Decrease	Decrease	↓Renal ROS production (cAMP and PKA)[102,179] ↓AGE–RAGE-mediated renal ROS production (cAMP)[181,265,266] ↓Angiotensin II-induced renal ROS production (PKC)[182,183] ↑Adiponectin (reduces podocyte inflammation; PKA in adipocytes)[267]	↑SDF1α[119,268,269] ↓Profibrotic endothelial-to-mesenchymal transition[185,186¶]
Glomerular hyperfiltration	Decrease or neutral effect	Neutral effect	↑Tubuloglomerular feedback (by ↓NHE3 activity) ↓Postprandial glucagon (particularly short-acting GLP-1RA)[70,71,90]? ↓Body weight[90]? ↓GEE* (postprandial hyperfiltration)[90]? ↓RAAS activity[87,127]?	↑SDF1α[119]?

ACE, angiotensin-converting enzyme; AGE, advanced glycation end products; BNP, brain natriuretic peptide; CNS, central nervous system; DPP-4, dipeptidyl peptidase 4; GEE, gastric emptying rate; GLP-1, glucagon-like peptide 1; GLP-1RA, GLP-1 receptor agonist; NHE3, sodium–hydrogen exchanger isoform 3; PKA, protein kinase A; PKC, protein kinase C; PYY, peptide YY; RAAS, renin–angiotensin–aldosterone system; RAGE, receptor for AGE; ROS, reactive oxygen species, SDF1α, stromal cell-derived factor 1α. *GEE reduction is subject to tachyphylaxis after prolonged treatment with long-acting GLP-1RA; however, loss of body weight continues[35,270]. ‡DPP-4 inhibition could blunt GLP-1-mediated effects on central regulation of satiation by concomitantly increasing levels of PYY (1–36), which increase appetite, and decreasing level of PYY (3–36), which decrease appetite. §Natriuresis seems to only be sustained with short-acting GLP-1RAs[116,117]; initial natriuresis with long-acting GLP-1RA may result in a new steady state with lower extracellular volume and/or lower sodium stores in the glycocalyx. ‖An ongoing trial is investigating this hypothesis in detail[223]. ¶This effect could be drug-specific as linagliptin, but not sitagliptin, reduces endothelial-to-mesenchymal transition[271].

also incompletely understood, but might include contributions from natriuresis and/or diuresis, improved vasorelaxation and/or endothelial function and neurohormonal pathways (TABLE 2).

Dyslipidaemia

Incretin-based therapies modestly contribute to improvement of fasting and particularly postprandial lipid profiles in patients with T2DM. A 2015 meta-analysis showed that GLP-1R agonist therapy results in small reductions in the levels of LDL cholesterol, total cholesterol and triglycerides, but does not improve HDL cholesterol levels in comparison to placebo and active comparators[165]. For DPP-4 inhibitors, a meta-analysis of 53 RCTs suggested a small benefit on total cholesterol levels of ~0.18 mmol/l (REF. 166). Mechanisms by which GLP-1R agonists improve dyslipidaemia are only partially understood, but clues from the regulation of postprandial lipid metabolism (fat ingestion is a stimulus of GLP-1 secretion) may be indicative. Evidence suggests that GLP-1R agonists reduce intestinal chylomicron production and secretion (contributing to reduced absorption and circulating levels of triglycerides), at least partly independent of changes in gastric emptying rate[167]. Incretin-based therapies reduced liver fat and decreased hepatic lipoprotein synthesis and secretion in some but not all studies[167,168]. Although these findings reflect a direct GLP-1-induced effect, indirect actions mediated by changes in circulating insulin and glucagon levels, neural inputs, weight loss, enhanced insulin sensitivity

or reduced substrate delivery seem more likely (TABLE 2). As such, incretin-based therapies may indirectly stimulate lipoprotein-lipase-mediated triglyceride uptake in white adipose tissue. Finally, GLP-1R agonists seem to facilitate clearance of lipids from the circulation by activating brown adipose tissue[169], which produces heat by burning triglycerides.

Inflammation, oxidative stress and fibrosis

The T2DM milieu drives a final common pathway of systemic and localized inflammation, oxidative stress and, eventually, proliferation and/or fibrosis that affects kidney function and morphology. Increasing evidence demonstrates that both pro-inflammatory and anti-inflammatory stimuli increase GLP-1 secretion, and GLP-1 in turn modulates inflammation at multiple sites, including the kidneys and blood vessels[167]. Preclinical studies report that incretin-based therapies inhibit inflammatory signalling pathways of DKD in a glucose-independent manner, commonly paralleled by reductions in albuminuria and improved histological features of diabetic nephropathy (Supplementary information S3 (table)). These include a study of DPP-4 inhibition plus angiotensin II receptor blocker in mice with knockout of endothelial nitric oxide synthase[170], a model which closely resembles human DKD[171,172]. Notably, in some studies that claimed glycaemic equipoise in intervention and placebo groups[102,173–178], glucose and/or HbA_{1c} levels were in fact slightly, albeit non-significantly, lower in the intervention group, which may have confounded the beneficial effect on inflammation.

The immunomodulatory actions of GLP-1R agonists might also be secondary to reductions in body weight. Nevertheless, observations in KK/Ta-Akita diabetic mice[102] and in rats with streptozotocin-induced diabetes[179] indicate that GLP-1R stimulation directly decreases levels of glomerular superoxide and renal NADPH oxidase through activation of cAMP and PKA. Moreover, exendin-4-induced stimulation of cAMP and PKA in human mesangial cells reduced proliferation and fibrosis[180]. GLP-1R-induced cAMP activation might also result in reduced expression of the receptor for advanced glycation end products, resulting in antioxidative effects[181]. The beneficial effects of GLP-1R signalling might also be mediated by counteracting angiotensin-II-induced NF-κB activation, as shown in glomerular endothelium and cultured mesangial cells[182,183].

In addition to GLP-1R-mediated effects[176], the glucose-independent anti-inflammatory and anti-fibrotic actions of DPP-4 inhibitors could be mediated by other DPP-4 substrates[17] or by direct mechanisms, as DPP-4 has roles in T cell development, T cell activation and immune regulation[58]. For example, the DPP-4 substrate SDF1α has a central role in cell and tissue repair under conditions of vascular occlusion and ischaemic renal injury[119]. Thus, linagliptin-induced expression of SDF1α in glomerular podocytes and distal nephrons was associated with reduced progression of albuminuria, glomerulosclerosis, periglomerular fibrosis, podocyte loss and renal oxidative stress in T2DM-prone mice[119] and rats[184]. Linagliptin also reduced renal fibrosis by directly inhibiting transforming growth factor-β (TGFβ)-induced endothelial-to-mesenchymal transition in T1DM mice models[185,186]. The mechanism could potentially involve suppression of hyperglycaemia-induced interactions of DPP-4 and cation-independent mannose-6-phosphate receptor[187] and/or DPP-4 and integrin β1 (REF. 188).

Glucose-independent anti-inflammatory and antioxidant actions of incretin-based therapies have also been described in patients with T2DM. However, the circulatory markers of inflammation and oxidative stress used in these studies might not reflect renal involvement. For example, 1 year of therapy with exenatide twice daily compared to insulin glargine[158] or glibenclamide[189] reduced the levels of high-sensitivity C-reactive protein by 25–60% in patients with T2DM, independent of glucose, body weight and body fat[158]. Treatment with liraglutide for 2 months improved markers of oxidative stress in patients with T2DM, independent of changes in fasting glucose or HbA_{1c} levels[190]. Finally, in microalbuminuric patients with T2DM, 16 weeks of treatment with exenatide twice daily reduced urinary excretion of TGFβ1 and type IV collagen compared to glimepiride, despite greater glycaemic improvements with glimepiride[191].

Incretin-based therapies and renal outcomes

GLP-1R agonists

The potential of incretin-based drugs to favourably affect renal risk factors in diabetes might translate into improved clinical outcomes, plausibly beyond glycaemic control. In uncontrolled studies involving patients with T2DM with or without nephropathy, liraglutide reduced albuminuria[137,138,192,193] and halted eGFR decline over a period of 12 months[192,193]. These results are partially in line with data from placebo-controlled RCTs of GLP-1R agonists in patients with T2DM. For example, in 846 overweight and obese patients with T2DM in the SCALE Diabetes trial[164], 56 weeks of treatment with liraglutide 3.0 mg, liraglutide 1.8 mg or placebo resulted in reductions in urine albumin-to-creatinine ratio (UACR) of 18.4%, 11.8% and 2.3%, respectively, suggesting dose-dependent effects of liraglutide on albuminuria. In the LIRA-RENAL trial[144], 279 patients with T2DM and moderate renal impairment (eGFR 30–59 ml/min/1.73 m^2) were randomly assigned to liraglutide 1.8 mg or placebo for 26 weeks. At study end, the intervention group showed a non-significant improvement in UACR relative to baseline (0.83, 95%-CI 0.62–1.10; $P = 0.19$) and no improvement in eGFR trajectory compared with placebo. Similarly, in patients with T2DM and normal renal function, 16–30 weeks of treatment with exenatide twice daily[148] and dulaglutide[149] did not affect eGFR over time.

Renal outcomes of placebo-controlled RCTs seldom characterize the impact of drug-induced differences in glycaemia or other renal risk factors. These differences were addressed, however, in a small crossover trial in albuminuric patients with T2DM on RAAS inhibitor therapy. Liraglutide treatment for 12 weeks reduced 24 h urinary albumin excretion by 32% compared with placebo[139], and multivariate regression showed that this

reduction was driven by a decrease in 24 h systolic blood pressure, and not by improvements in HbA_{1c} levels, body weight or GFR[139].

Head-to-head trials of GLP-1R agonists with active comparators can directly determine glucose-independent effects on renal end points. A retrospective analysis that propensity-score-matched baseline HbA_{1c} levels showed that 1 year of treatment with exenatide twice daily versus insulin glargine reduced UACR by 104 mg/g versus 47 mg/g, although the between-group differences were not significant[194]. Integrated data from nine registration trials of dulaglutide, which included 6,005 patients with T2DM, showed lower UACRs with this GLP-1R agonist than with placebo (−16.7% versus −10.0%; $P = 0.043$), insulin glargine (−16.7% versus −3.7%; $P = 0.127$) and other active comparators (−20.0% versus −12.5%; $P = 0.054$)[149]. No significant differences in serum creatinine levels or eGFR were observed over a 26–104 week period, but fewer patients who received dulaglutide than those who received insulin glargine experienced a 40% decline in eGFR at any point during a 1-year treatment period (0.26% versus 1.25%; $P = 0.012$)[149]. The available data from the 26-week AWARD-7 trial in patients with T2DM and moderate-to-severe CKD receiving insulin lispro, suggest that dulaglutide treatment compared with insulin glargine reduced albuminuria and might have also had a beneficial effect on eGFR decline, particularly in those with UACR >300 mg/g (REF. 195). Moreover, in patients with T2DM, 26 weeks of exenatide therapy reduced HbA_{1c} levels to a similar extent as glimepiride, but resulted in a greater decrease in 24 h urinary albumin excretion (38.0% versus 5.8%)[191]. However, an 8-week trial of lixisenatide versus titrated once-daily insulin glulisine in insulin glargine-treated patients with T2DM without overt nephropathy did not show an HbA_{1c}-independent renal benefit of the GLP-1R agonist[117].

DPP-4 inhibitors

Increased serum and renal DPP-4 activity is associated with albuminuria and DKD[196–201]. Conversely, uncontrolled studies reported reductions in albuminuria after DPP-4 inhibitor treatment for 12 weeks to 1 year in patients with T2DM with and without nephropathy[202–205]. A pooled analysis of 13 placebo-controlled RCTs of linagliptin, which included 5,466 patients with inadequately controlled T2DM, showed that the intervention reduced kidney disease events by 16% (95% CI 3–28%; $P = 0.02$) (REF. 147). This outcome was driven by an 18% reduction in moderate and 14% reduction in severe elevations of albuminuria with no significant effects on eGFR or incidence of acute kidney injury (AKI). Moreover, combined data from four RCTs in 217 albuminuric patients with T2DM on RAAS inhibition indicated that linagliptin reduced UACR by 28% versus placebo, independent of HbA_{1c} levels or systolic blood pressure[206]. The MARLINA-T2D™ trial in 360 patients with T2DM on stable RAAS inhibition[207], which was sufficiently powered to test the superiority of linagliptin versus placebo on predefined albuminuria, could not confirm these findings. However, analysis by responder categories showed an approximately 70% higher rate of achieving a >20% reduction in UACR in the linagliptin group than in the placebo group, which merits further study[207]. The CARMELINA trial[208], which includes a composite renal outcome of hard renal end points as a key secondary objective, will determine whether long-term linagliptin therapy has renal effects in a T2DM population that includes 7,003 patients with more advanced CKD; results are expected in 2018.

In an open-label RCT in 85 patients with T2DM, sitagliptin compared to other glucose-lowering drugs achieved similar HbA_{1c} reductions and significantly decreased log-UACR by 23.3% after 6 months, without affecting eGFR[209]. Interestingly, the sitagliptin-induced UACR reduction correlated with changes in diastolic blood pressure, but not in HbA_{1c} levels[209]. Finally, a RCT in 170 patients with T2DM showed that sitagliptin and exenatide once weekly both reduced UACR compared to pioglitazone after 26 weeks[210].

Outcomes of cardiovascular safety studies

Licencing of antihyperglycaemic drugs is subject to the 2008 US FDA industry guidance, which requires robust cardiovascular safety data in high-risk T2DM as a prerequisite for drug approval[211]. Six large randomized cardiovascular outcome studies involving incretin-based therapies have now reported their primary results (Supplementary information S4 (figure)). Although the design and primary aim of most cardiovascular outcome studies is to establish non-inferiority to placebo (that is, standard diabetes care) in terms of major adverse cardiovascular events (MACE), all of these studies incorporated secondary and exploratory renal end points (TABLE 3).

GLP-1 receptor agonists

Cardiovascular outcomes. The 2015 ELIXA trial[212] assessed the cardiovascular safety of lixisenatide versus placebo in 6,068 patients with T2DM and an acute coronary event <180 days before screening. No significant differences were observed in rates of four-point MACE (a composite of cardiovascular death, nonfatal myocardial infarction (MI), nonfatal stroke or hospitalization for unstable angina), or hospitalization for heart failure. Given the fairly short follow-up period (~25 months) and strict enrolment criteria, no conclusions can be drawn regarding the cardioprotective potential of long-term lixisenatide in other T2DM populations.

The cardiovascular safety of ~3.8 years of liraglutide therapy was examined in the LEADER trial[65], which included 9,340 patients with T2DM and high cardiovascular risk. In this study, liraglutide therapy was associated with a significant 13% reduction in the first occurrence of three-point MACE (cardiovascular death, nonfatal MI or nonfatal stroke), comprising a 22% reduction in cardiovascular death and a trend towards lower frequencies of nonfatal MI (12%) and nonfatal stroke (11%). Based on the LEADER data, 66 patients with T2DM would need to be treated with liraglutide for 3 years to prevent one cardiovascular event[65].

Subsequently, the SUSTAIN-6 trial[213] reported that 2.1 years of semaglutide therapy not only achieved non-inferiority to placebo, but also demonstrated a 26%

Table 3 | **Renal outcomes in CV safety studies of incretin-based therapies in high-risk patients with type 2 diabetes mellitus**

Trial (year)	Drug	Patients (*n*)	Risk factors at baseline			Follow-up	Renal end point	Exploratory renal outcome (change from baseline placebo/intervention or HR 95% CI)	Refs
			HbA_{1c} (%)	BP (mmHg)	RAAS inhibitor (%)				
GLP-1 receptor agonists									
ELIXA (2015)	Lixisenatide	CV 6,068; renal 5,633	7.7	129.5/70.2	85	CV 25 months; renal 24 months	Change in UACR (%)	Month 24: 34/24*‡	212
LEADER (2016)	Liraglutide	9,340	8.7	135.9/77.1	83	3.8 years	Time to primary composite end point	HR 0.78 (0.67–0.92)*	65,66
							Time to new onset of persistent MA	HR 0.74 (0.60–0.91)*	
							Persistent doubling of SCr§	HR 0.89 (0.67–1.19)	
							Need for continuous RRT	HR 0.87 (0.61–1.24)	
							Death due to renal disease	HR 1.59 (0.52–4.87)	
SUSTAIN-6 (2016)	Semaglutide	3,297	8.7	135.7/77.1	84	2.1 years	New or worsening nephropathy	HR 0.64 (0.46–0.88)*	213
							Persistent MA	HR 0.54 (0.37–0.77)*	
							Persistent doubling of SCr‖	HR 1.28 (0.64–2.58)	
							Need for continuous RRT	HR 0.91 (0.40–2.07)	
DPP-4 inhibitors									
SAVOR-TIMI 53 (2013)	Saxagliptin	CV 16,492; renal 15,760	8.0	NR	82	2.1 years	Improved UACR (%)	• Year 1: 9.6/11.8* • Year 2: 9.2/11.1* • Study end: 8.7/10.7*	215,224
							Worsened UACR (%)	• Year 1: 11.4/9.4* • Year 2: 14.2/12.4* • Study end: 15.9/13.3*	
							Change in UACR (mg/g)	Study end: −34.3*	
							Change in UACR category	Study end: lowered by saxagliptin*	
							Doubling of SCr	HR 1.1 (0.89–1.36)	
							Initiation of RRT¶	HR 0.90 (0.61–1.32)	
EXAMINE (2013)	Alogliptin	CV 5,380; renal 839	8.0	NR	82	18 months	Change in eGFR (ml/min/1.73 m^2)	• eGFR >90: −4.5/−6.7 • eGFR 60–90: 1.0/0.6 • eGFR 30–60: 2.1/1.1 • eGFR <30: 1.6/0.2	216
TECOS (2015)	Sitagliptin	CV 14,671; eGFR 13,604; UACR 3,832	7.2	135.0/77.2	79	CV 3 years; renal 4 years	Microalbuminuria (%)	Year 3: 7.9/7.8	217,225
							Renal failure (%)	Year 3: 1.5/1.4	
							Mean difference in eGFR (ml/min/1.73 m^2)	Year 4: −1.34 (−1.76 to −0.91)#	
							Mean difference in UACR (mg/g)	Year 4: −0.18 (−0.35 to −0.02)	

*Statistically significant. ‡Statistically significant after adjustment for baseline, treatment, region, baseline use of ACE inhibitor and ARB ($P<0.01$). $P=0.07$ after *post hoc* adjustment for both baseline and 3-month HbA_{1c} levels (ACR placebo +32%, lixisenatide +26%). §Persistent doubling of serum creatinine level and eGFR ≤45 ml/min/1.73 m^2 using MDRD. ‖Persistent doubling of serum creatinine level and eGFR <45 ml/min/1.73 m^2 using MDRD. ¶And/or creatinine >6.0 mg/dl (530 µmol/l). #Similar results according to baseline eGFR stage. Difference remained after adjustment for time from randomization when eGFR was measured, baseline eGFR, baseline HbA_{1c}, change in HbA_{1c} and region (−1.43 ml/min/1.73 m^2, 95% CI −1.88 to 0.98). **Similar results according to baseline eGFR stage. BP, blood pressure; CV, cardiovascular; DPP-4, dipeptidyl peptidase 4; eGFR, estimated glomerular filtration rate; GLP-1, glucagon-like peptide 1; MA, macroalbuminuria; MDRD, Modification of Diet in Renal Disease equation; RAAS, renin-angiotensin-aldosterone system; RRT, renal replacement therapy; SCr, serum creatinine; UACR, urinary albumin:creatinine ratio.

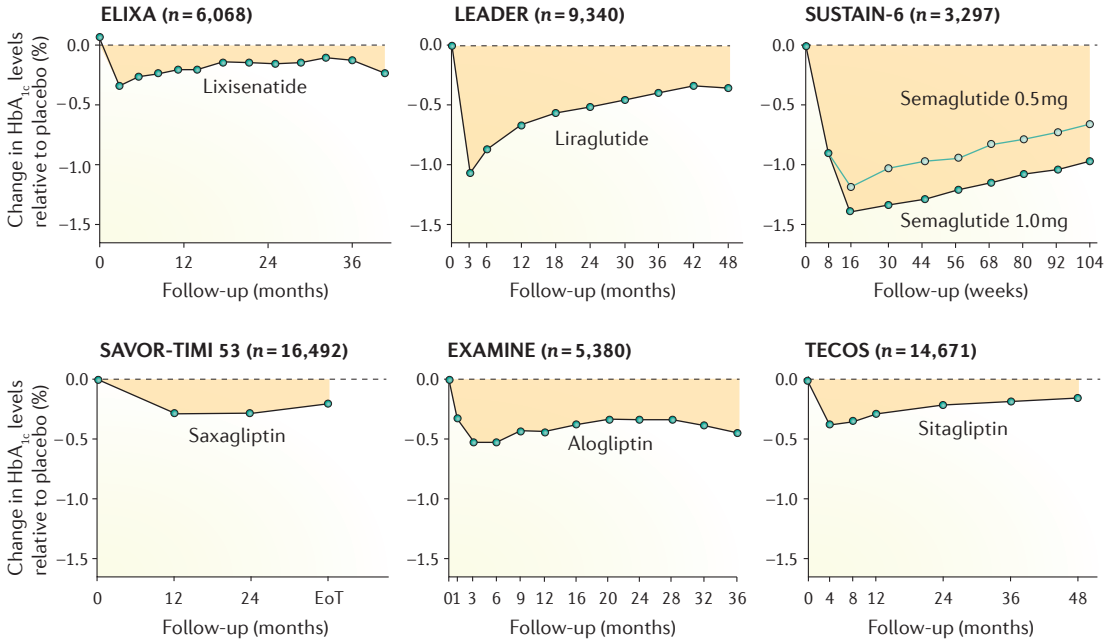

Figure 6 | **Differences in glycaemic control with the study drug versus placebo in cardiovascular outcome studies assessing the safety of incretin-based therapies in patients with type 2 diabetes mellitus at high risk of cardiac events.** Data obtained from Pfeffer *et al.* (ELIXA)[212], Marso *et al.* (LEADER)[65], Marso *et al.* (SUSTAIN-6)[213], Scirica *et al.* (SAVOR-TIMI 53)[215], White *et al.* (EXAMINE)[216] and Green *et al.* (TECOS)[217]. EoT, end of treatment.

relative risk reduction in three-point MACE in 3,297 patients with T2DM and high cardiovascular risk. Unlike LEADER, SUSTAIN-6 did not show a significant benefit of the intervention on cardiovascular death as an individual secondary end point, but did demonstrate a 26% reduction in nonfatal MI and a 39% reduction in nonfatal stroke.

Renal outcomes. In ELIXA, the prespecified analysis of the percentage change in UACR (measured from baseline to 108 weeks of treatment) showed a modest difference in favour of lixisenatide over placebo (24% versus 34%; $P = 0.004$). *Post hoc* adjustment for slight differences in HbA_{1c} levels (~0.3%) during the first 3 months of the trial attenuated the lixisenatide-induced renal benefit ($P = 0.07$), suggesting some glucose-dependency[212].

Both LEADER and SUSTAIN-6 employed a prespecified composite outcome of new or worsening nephropathy consisting of mostly adjudicated events defined as new onset of persistent macroalbuminuria; persistent doubling of serum creatinine level and eGFR ≤45 ml/min/1.73m^2; need for continuous renal replacement therapy (in the absence of an acute reversible cause); and renal death. Liraglutide reduced new or worsening nephropathy by 22% in LEADER after 3.8 years[65,66], whereas 104 weeks of semaglutide treatment reduced this composite outcome by 36%[213]. Notably, the renal benefit in LEADER was predominantly driven by a 26% reduction in macroalbuminuria, whereas in SUSTAIN-6, an even larger 46% reduction in macroalbuminuria seems exclusively responsible for the favourable renal outcome of semaglutide[213].

Given the glucose-dependency of the albuminuria effect in ELIXA, and the fact that differences in HbA_{1c} levels in LEADER and SUSTAIN-6 were even more substantial (time-averaged mean compared to placebo of ~0.6% and ~1.1%, respectively (FIG. 6)), the potential impact of glycaemic control should be recognized[214]. As neither of the trials hitherto reported *post hoc* adjustments for differences in HbA_{1c} levels on their renal outcomes, nor investigated the contributions of modest improvements in body weight (~2–4 kg) and systolic blood pressure (~1–3 mmHg), the drug-specific renal benefits of liraglutide and semaglutide remain uncertain. Finally, in LEADER, liraglutide slowed eGFR decline over time compared to placebo, with a baseline to month 36 ratio of 0.89 with liraglutide and 0.88 with placebo (difference of 1.015, $P = 0.013$). Subgroup analyses revealed that this decline occurred primarily in patients with eGFR 30–59 ml/min/1.73m^2 at baseline[66].

DPP-4 inhibitors

Cardiovascular outcomes. SAVOR-TIMI 53 (REF. 215), EXAMINE[216] and TECOS[217] examined the cardiovascular safety of the DPP-4 inhibitors saxagliptin ($n = 16,492$), alogliptin ($n = 5,380$) and sitagliptin ($n = 14,671$) for 2.1 years, 1.5 years and 3 years, respectively. These cardiovascular outcome studies were conducted mainly in patients with T2DM at high risk of cardiovascular events. All three trials achieved the non-inferiority margins specified by the FDA in their three-point MACE (saxagliptin and alogliptin) or four-point MACE (sitagliptin), suggesting that the drugs are cardiovascular neutral.

Notably, SAVOR-TIMI 53 found a significant 27% increase in hospitalization for heart failure (particularly <1 year after randomization) with saxagliptin versus placebo, whereas a nonsignificant 19%

increase in this outcome was observed with alogliptin in EXAMINE, and no signal was found with sitagliptin in TECOS. A recent meta-analysis[218] of these cardiovascular outcome studies and other DPP-4 inhibitor trials concluded that only saxagliptin significantly increases heart failure risk, particularly among patients with T2DM and previous heart failure, increased natriuretic peptide levels or CKD[219]. Data from mechanistic studies suggest that this increased risk might partly result from an unfavourable interaction between DPP-4 inhibition and high-dose ACE inhibition, which could potentially increase the levels and activity of substance P and NPY, resulting in augmentation of sympathetic activity, heart rate and vascular tone[127,220]. Some reassurance was provided, however, by *post hoc* analyses of SAVOR-TIMI 53 (REF. 221) and EXAMINE[222], which did not show an effect of DPP-4 plus ACE inhibition on cardiovascular or heart failure risk. The results of a dedicated mechanistic trial that is investigating the interaction between DPP-4 inhibition and high-dose ACE inhibition in 150 hypertensive patients with T2DM are expected in 2018[223].

Renal outcomes. In SAVOR-TIMI 53, more patients with T2DM in the saxagliptin group than in the placebo group shifted to a lower UACR category, irrespective of baseline UACR category ($P = 0.021$ for normoalbuminuria, $P < 0.001$ for microalbuminuria and $P = 0.049$ for macroalbuminuria)[215,224]. An overall mean reduction in UACR of 34 mg/g ($P < 0.004$) was seen with saxagliptin, mainly owing to improvements in UACR in patients with macroalbuminuria[215,224]. UACR also decreased more in patients with lower baseline eGFR than in those with better renal function at baseline (eGFR >50 ml/min/1.73 m^2, −19 mg/g; eGFR 30–50 ml/min/1.73 m^2, −105 mg/g; eGFR<30 ml/min/1.73 m^2, −245 mg/g). Very weak correlations (Pearson coefficients ≤0.052) between changes in UACR and HbA_{1c} levels were reported for the entire study population and for the separate treatment groups[224]. Moreover, similar reductions in albuminuria were observed in patients with HbA_{1c} reductions of ≥0.5% and <0.5% after 2 years. Early improvements in glycaemia with saxagliptin were not accounted for in these analyses (TABLE 3).

EXAMINE only reported change in eGFR from baseline, which was similar in the alogliptin and placebo groups after 18 months of therapy[216]. In TECOS, eGFR declined at the same rate in both treatment groups over a 4-year period but was slightly higher (1.34 ml/min/1.73 m^2) in the sitagliptin group than in the placebo group[217,225]. This difference occurred in the first 4 months after randomization, was similar across eGFR categories and was sustained after adjustment for baseline eGFR and HbA_{1c} levels[225]. UACR (which was measured in only 26% of patients) was marginally lower in the sitagliptin group (−0.18 mg/g, 95% CI −0.35 to −0.02) than in the placebo group, with no significant interaction of treatment effect by eGFR stage. Whether such small sitagliptin-induced offsets in eGFR and UACR would have long-term clinical implications is unclear.

Tolerability and safety

Incretin-based therapies are generally well tolerated. The incidence of adverse effects with DPP-4 inhibitors is similar to placebo and is lower than for other antihyperglycaemic drugs[13]. The incidence of adverse events with GLP-1R agonists is, however, higher than with DPP-4 inhibitors[226]. As antihyperglycaemic actions of incretin-based therapies are glucose-dependent, the risk of hypoglycaemia is very low, except when these agents are combined with insulin or sulfonylureas.

Nausea

The most common adverse effect of GLP-1R agonists (but not DPP-4 inhibitors) is nausea, with incidence rates varying from 25–60%, and vomiting in 5–15%. Although nausea is usually transient, resolves over 4–8 weeks, and can be minimized by gradually increasing the dose, it remains present in 9% of patients treated with short-acting and 3% of those treated with long-acting GLP-1R agonists, and 5–10% of patients typically discontinue the drug[68,69]. The mechanisms that underlie these gastrointestinal complaints remain uncertain, but might reflect an aversive response or reduced gastric emptying.

Pancreatitis and pancreatic cancer

The putative association between incretin-based therapies, acute pancreatitis and pancreatic cancer has received much attention, but no definite causal link has been found to date[38]. A 2017 meta-analysis that included data from all available trials with a minimum treatment duration of 24 months, found no link between acute pancreatitis and GLP-1R agonists (OR 0.745, 95% CI 0.47–1.17)[227], whereas DPP-4 inhibitors significantly increased the risk of acute pancreatitis relative to placebo (RR 1.79, 95% CI 1.13–2.81)[228]. Given the low incidence of acute pancreatitis in patients with T2DM, DPP-4 inhibitor treatment causes 5.5 extra cases per 10,000 patients per year, with a number needed to harm of 1,940 patients a year.

Concerns regarding pancreatic cancer are not supported either by RCTs (including cardiovascular outcome studies) or by a population-based cohort study that included almost 1 million patients initiating antihyperglycaemic drugs (HR 1.02, 95%-CI 0.84–1.23)[229]. Controversy remains, however, because pancreatic cancer takes years to develop, and the duration of follow-up and numbers of participants in current trials are insufficient to draw definite conclusions.

Acute kidney injury

Initial case reports[17] and a nested case-control study in 13,504 patients with T2DM receiving DPP-4 inhibitors with <1 year of follow-up[230] suggested that incretin-based therapies may increase AKI risk. This finding was not confirmed by a retrospective database study[231], prospective registration studies[147–149] or cardiovascular outcome studies[66,224]. Conversely, in line with the renoprotective mechanisms outlined above, DPP-4 inhibitors[232–237] and GLP-1R agonists[233,238] have been reported to protect against AKI in animal models. In addition, a large prospective cohort study with 3.6 years of follow-up in

923,936 patients with diabetes reported reduced AKI risk in those who received DPP-4 inhibitors[239]. However, given that GLP-1R signalling has natriuretic and diuretic properties and could increase afferent arteriolar resistance through tubuloglomerular feedback, healthcare providers might need to consider factors that predispose to AKI prior to initiating therapy or upon monitoring of the drug in select cases.

Cholecystitis and gall stones

Increased risk of cholecystitis and acute gallstone disease (requiring cholecystectomy) in patients receiving liraglutide was reported in LEADER[65] and SCALE[156], but was not seen with other GLP-1R agonists or DPP-4 inhibitors. Rapid weight loss might only partly explain higher rates of gallbladder events with liraglutide, as excess risk was seen across all weight-loss categories[65,156]. Although acute exenatide administration reduces gallbladder emptying rate in healthy individuals[240], we found no such effect of 12-week liraglutide in patients with T2DM[241].

Retinopathy

Increased rates of retinopathy events were seen in patients with T2DM receiving semaglutide in SUSTAIN-6 (HR 1.76, 95% CI 1.11–2.78)[213], and retinopathy rates also tended to increase with liraglutide therapy in LEADER[65]. Data from seminal T1DM[242] and, to a lesser extent, T2DM studies[243], together with the finding that retinopathy events occurred early after treatment initiation in the SUSTAIN-6 trial, suggest that intense and rapid glucose-lowering with semaglutide might underlie this adverse effect, rather than a drug-specific effect[213]. Indeed, in both the semaglutide and placebo groups, the highest rates of retinopathy events were among patients who experienced a >1.5% drop in HbA_{1c} levels, whereas the lowest rates were among patients who experienced reductions of <0.5%[213,244].

Uncertainties and future perspectives

The finding in pooled analyses and cardiovascular outcome studies that incretin-based therapies reduce the risk of albuminuria, as well as the report that liraglutide therapy marginally reduced eGFR decline in the LEADER trial, highlight the need to further refine and extend current understanding of the underlying mechanisms. Most trials did not, however, properly adjust renal outcome for impact of glycaemic differences. *Post hoc* analyses of LEADER and SUSTAIN-6 are now highly anticipated to ascertain non-glycaemic, drug-specific benefits[214], as was elegantly done for the EMPA-REG OUTCOME trial data, which showed a glucose-independent beneficial effect of the SGLT2 inhibitor empagliflozin on UACR[245,246]. Weight loss and blood pressure reductions could account for the modest renal benefits with GLP-1R agonists and, to lesser extent, with DPP-4 inhibitors. The relative importance of improved postprandial lipid levels, reduced systemic or tissue inflammation and possibly direct renal effects (that is, natriuresis and potentially small reductions in glomerular hyperfiltration) will likely be impossible to assess. Futhermore, ensuring glycaemic equipoise in future studies is of paramount importance to abolish the need for *post hoc* correction, and these trials should directly compare clinical effects with existing treatments. The ongoing CAROLINA (linagliptin versus glimepiride[247])and GRADE trials (comparing liraglutide, sitagliptin, glimepiride and insulin glargine[248])stand out in this regard, although the results are not expected until 2019 and 2020, respectively, and the renal outcomes are exploratory end points.

In general, incretin-based therapies improve albuminuria, but this outcome is regarded as a potential surrogate renal end point[4], and effects on clinically relevant renal outcomes remain uncertain. In contrast to SGLT2 inhibitors, no ongoing studies of incretin-based drugs in at-risk patients with T2DM (or in obese individuals or nondiabetic patients with CKD) have renal end points as a primary objective or seem to be of sufficient duration to show an effect on clinically relevant renal outcomes. As such, uncertainties surrounding the glucose-independent benefits of these drugs on hard renal end points will endure for years to come.

Combination treatment with incretin-based therapies and SGLT2 inhibitors, which improve glycaemia by inducing glycosuria[16], could potentially have beneficial effects on glycaemic control and renal risk factors that are superior to those achieved with either drug alone. For example, it has been suggested that GLP-1R agonists might suppress the adaptive hyperphagia that is associated with long-term SGLT2 inhibitor therapy and results in substantially less weight loss than would be expected based on the energy lost via glycosuria[249]. The findings of the DURATION-8 trial[250], which showed an additive effect on weight loss with dapagliflozin plus exenatide once-weekly versus either drug alone after 28 weeks of therapy, support this hypothesis. It is tempting to speculate that additive or synergistic actions of incretin-based therapies and SGLT2 inhibitors in patients with T2DM also encompass blood pressure, LDL cholesterol, natriuresis and perhaps renal haemodynamics[251]. As such, an important outstanding question in future trials will be whether combinations of these drug classes have greater renal and cardiovascular benefits than the individual drugs or than intensive therapy with other glucose-lowering agents.

Conclusions

Among the numerous peptides secreted from enteroendocrine cells, GLP-1 has become the most extensively studied gut hormone with the greatest translational relevance in terms of pathophysiological discoveries and drug development. After more than a decade of use of incretin-based therapies, the understanding and value of therapeutically engaging GLP-1R-mediated mechanisms for T2DM treatment is becoming increasingly clear. In addition to glucose-lowering, incretin-based therapies enable maintenance of or reductions in body weight, blood pressure and lipid levels. Such effects, which are not achieved by current standard diabetes care, might help to narrow the gap between recommended and established risk factor control in clinical

practice. Furthermore, in line with emerging data that support the existence of a rapid-acting gut–renal axis, GLP-1 and associated therapies increase the renal excretion of electrolytes (most notably sodium), and might under certain circumstances ameliorate glomerular hyperfiltration in patients with T2DM. Clinical data of pooled registration trials and results of large-sized cardiovascular outcome studies indicate that use of GLP-1R agonists and, to a lesser extent, DPP-4 inhibitors in addition to standard care modestly improve albuminuria in T2DM, plausibly beyond the effects of glycaemic control. Liraglutide also marginally halted eGFR decline in patients with T2DM who were at high risk of cardiovascular and renal events. The renal benefits of incretin-based therapies do, however, seem to be less pronounced than those of SGLT2 inhibitors.

The development of incretin-based therapies provides a model for harnessing the pharmacological potential of entero-endocrine-cell-derived hormones. In future decades, more gut-derived molecules that are involved in the regulation of renal physiology will likely be discovered. Such molecules might provide new treatment strategies to prevent the onset and progression of DKD or of renal disease in general.

1. International Diabetes Federation. IDF Diabetes Atlas 7th edn. *Diabetes Atlas* http://www.diabetesatlas.org/ (2015).
2. King, H., Aubert, R. E. & Herman, W. H. Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. *Diabetes Care* **21**, 1414–1431 (1998).
3. Narayan, K. M., Boyle, J. P., Geiss, L. S., Saaddine, J. B. & Thompson, T. J. Impact of recent increase in incidence on future diabetes burden: U. S., 2005–2050. *Diabetes Care* **29**, 2114–2116 (2006).
4. Muskiet, M. H. *et al.* Pleiotropic effects of type 2 diabetes management strategies on renal risk factors. *Lancet Diabetes Endocrinol.* **3**, 367–381 (2015).
5. Nathan, D. M. Diabetes: advances in diagnosis and treatment. *JAMA* **314**, 1052–1062 (2015).
6. Gregg, E. W. *et al.* Changes in diabetes-related complications in the United States, 1990–2010. *N. Engl. J. Med.* **370**, 1514–1523 (2014).
7. Gregg, E. W., Sattar, N. & Ali, M. K. The changing face of diabetes complications. *Lancet Diabetes Endocrinol.* **4**, 537–547 (2016).
8. Thomas, M. C., Cooper, M. E. & Zimmet, P. Changing epidemiology of type 2 diabetes mellitus and associated chronic kidney disease. *Nat. Rev. Nephrol.* **12**, 73–81 (2016).
Review of the epidemiology of T2DM and CKD in various regions worldwide, reporting that the decreasing prevalence of cardiovascular disease results in increased survival, which translates to an increased prevalence of renal complications.
9. Fernandez-Fernandez, B., Ortiz, A., Gomez-Guerrero, C. & Egido, J. Therapeutic approaches to diabetic nephropathy — beyond the RAS. *Nat. Rev. Nephrol.* **10**, 325–346 (2014).
10. Fried, L. F. *et al.* Combined angiotensin inhibition for the treatment of diabetic nephropathy. *N. Engl. J. Med.* **369**, 1892–1903 (2013).
11. European Medicine Agency. Combined use of medicines affecting the renin-angiotensin system (RAS) to be restricted — CHMP endorses PRAC recommendation. *EMA* http://www.ema.europa.eu/docs/en_GB/document_library/Press_release/2014/05/WC500167421.pdf (2014).
12. Inzucchi, S. E. *et al.* Management of hyperglycemia in type 2 diabetes, 2015: a patient-centered approach: update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care* **38**, 140–149 (2015).
13. Tahrani, A. A., Barnett, A. H. & Bailey, C. J. Pharmacology and therapeutic implications of current drugs for type 2 diabetes mellitus. *Nat. Rev. Endocrinol.* **12**, 566–592 (2016).
Comprehensive review of currently available glucose-lowering therapies for the treatment of T2DM, focusing on mechanisms of action, pharmacokinetics, pharmacodynamics and safety profiles.
14. DeFronzo, R. A., Norton, L. & Abdul-Ghani, M. Renal, metabolic and cardiovascular considerations of SGLT2 inhibition. *Nat. Rev. Nephrol.* **13**, 11–26 (2017).
15. Heerspink, H. J., Perkins, B. A., Fitchett, D. H., Husain, M. & Cherney, D. Z. Sodium glucose cotransporter 2 inhibitors in the treatment of diabetes mellitus: cardiovascular and kidney effects, potential mechanisms, and clinical applications. *Circulation* **134**, 752–772 (2016).
16. van Bommel, E. J. *et al.* SGLT2 inhibition in the diabetic kidney — from mechanisms to clinical outcome. *Clin. J. Am. Soc. Nephrol.* (2017).
17. Muskiet, M. H., Smits, M. M., Morsink, L. M. & Diamant, M. The gut-renal axis: do incretin-based agents confer renoprotection in diabetes? *Nat. Rev. Nephrol.* **10**, 88–103 (2014).
First review of the potential renoprotective effects and mechanisms of GLP-1R agonists and DPP-4 inhibitors in preclinical studies and in early clinical studies involving patients with diabetes.
18. Drucker, D. J. Evolving concepts and translational relevance of enteroendocrine cell biology. *J. Clin. Endocrinol. Metab.* **101**, 778–786 (2016).
Excellent review of the complexity of entero-endocrine cell biology, discussing gaps in current understanding and highlighting the translational relevance of entero-endocrine cells in the pathophysiology and therapy of metabolic and inflammatory disorders.
19. Furness, J. B., Rivera, L. R., Cho, H. J., Bravo, D. M. & Callaghan, B. The gut as a sensory organ. *Nat. Rev. Gastroenterol. Hepatol.* **10**, 729–740 (2013).
20. Gribble, F. M. & Reimann, F. Enteroendocrine cells: chemosensors in the intestinal epithelium. *Annu. Rev. Physiol.* **78**, 277–299 (2016).
21. La Barre, J. Sur les possibilités d'un traitement du diabète par l'incrétine. *Bull. Acad. R. Med. Belg.* **12**, 620–634 (1932).
22. Nauck, M. A. & Meier, J. J. The incretin effect in healthy individuals and those with type 2 diabetes: physiology, pathophysiology, and response to therapeutic interventions. *Lancet Diabetes Endocrinol.* **4**, 525–536 (2016).
23. Nauck, M. A. *et al.* Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. *J. Clin. Endocrinol. Metab.* **63**, 492–498 (1986).
24. Perley, M. J. & Kipnis, D. M. Plasma insulin responses to oral and intravenous glucose: studies in normal and diabetic sujbjects. *J. Clin. Invest.* **46**, 1954–1962 (1967).
25. Buchan, A. M., Polak, J. M., Capella, C., Solcia, E. & Pearse, A. G. Electronimmunocytochemical evidence for the K cell localization of gastric inhibitory polypeptide (GIP) in man. *Histochemistry* **56**, 37–44 (1978).
26. Eissele, R. *et al.* Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man. *Eur. J. Clin. Invest.* **22**, 283–291 (1992).
27. Cho, Y. M., Fujita, Y. & Kieffer, T. J. Glucagon-like peptide-1: glucose homeostasis and beyond. *Annu. Rev. Physiol.* **76**, 535–559 (2014).
28. Smits, M. M. *et al.* Gastrointestinal actions of glucagon-like peptide-1-based therapies: glycaemic control beyond the pancreas. *Diabetes Obes. Metab.* **18**, 224–235 (2016).
29. Nauck, M. A. *et al.* Effects of glucagon-like peptide 1 on counterregulatory hormone responses, cognitive functions, and insulin secretion during hyperinsulinemic, stepped hypoglycemic clamp experiments in healthy volunteers. *J. Clin. Endocrinol. Metab.* **87**, 1239–1246 (2002).
30. Schirra, J. *et al.* Exendin(9–39)amide is an antagonist of glucagon-like peptide-1(7–36)amide in humans. *J. Clin. Invest.* **101**, 1421–1430 (1998).
31. Salehi, M., Vahl, T. P. & D'Alessio, D. A. Regulation of islet hormone release and gastric emptying by endogenous glucagon-like peptide 1 after glucose ingestion. *J. Clin. Endocrinol. Metab.* **93**, 4909–4916 (2008).
32. de Heer, J., Rasmussen, C., Coy, D. H. & Holst, J. J. Glucagon-like peptide-1, but not glucose-dependent insulinotropic peptide, inhibits glucagon secretion via somatostatin (receptor subtype 2) in the perfused rat pancreas. *Diabetologia* **51**, 2263–2270 (2008).
33. Meier, J. J. *et al.* Gastric inhibitory polypeptide (GIP) dose-dependently stimulates glucagon secretion in healthy human subjects at euglycaemia. *Diabetologia* **46**, 798–801 (2003).
34. Meier, J. J., Deacon, C. F., Schmidt, W. E., Holst, J. J. & Nauck, M. A. Suppression of glucagon secretion is lower after oral glucose administration than during intravenous glucose administration in human subjects. *Diabetologia* **50**, 806–813 (2007).
35. van Bloemendaal, L., Ten Kulve, J. S., la Fleur, S. E., Ijzerman, R. G. & Diamant, M. Effects of glucagon-like peptide 1 on appetite and body weight: focus on the CNS. *J. Endocrinol.* **221**, T1–T16 (2014).
Review describing the physiological role of GLP-1 in the central regulation of feeding behaviour, as well the effects of pharmacological stimulation of GLP-1 pathways leading to alterations in CNS activity, reduced food intake and weight loss in obesity and T2DM.
36. Ten Kulve, J. S. *et al.* Liraglutide reduces cns activation in response to visual food cues only after short-term treatment in patients with type 2 diabetes. *Diabetes Care* **39**, 214–221 (2016).
37. van Bloemendaal, L. *et al.* GLP-1 receptor activation modulates appetite- and reward-related brain areas in humans. *Diabetes* **63**, 4186–4196 (2014).
38. Smits, M. M. *et al.* GLP-1 based therapies: clinical implications for gastroenterologists. *Gut* **65**, 702–711 (2016).
39. Nerup, N. *et al.* The effect of glucagon-like peptide-1 and glucagon-like peptide-2 on microcirculation: a systematic review. *Microcirculation* http://dx.doi.org/10.1111/micc.12367 (2017).
40. Nauck, M., Stockmann, F., Ebert, R. & Creutzfeldt, W. Reduced incretin effect in type 2 (non-insulin-dependent) diabetes. *Diabetologia* **29**, 46–52 (1986).
First study that showed that the incretin effect is markedly reduced in patients with T2DM.
41. Knop, F. K. *et al.* Reduced incretin effect in type 2 diabetes: cause or consequence of the diabetic state? *Diabetes* **56**, 1951–1959 (2007).
42. Bagger, J. I. *et al.* Impaired regulation of the incretin effect in patients with type 2 diabetes. *J. Clin. Endocrinol. Metab.* **96**, 737–745 (2011).
43. Kahn, S. E., Cooper, M. E. & Del Prato, S. Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future. *Lancet* **383**, 1068–1083 (2014).
44. Calanna, S. *et al.* Secretion of glucose-dependent insulinotropic polypeptide in patients with type 2 diabetes: systematic review and meta-analysis of clinical studies. *Diabetes Care* **36**, 3346–3352 (2013).
45. Calanna, S. *et al.* Secretion of glucagon-like peptide-1 in patients with type 2 diabetes mellitus: systematic review and meta-analyses of clinical studies. *Diabetologia* **56**, 965–972 (2013).
46. Faerch, K. *et al.* GLP-1 response to oral glucose is reduced in prediabetes, screen-detected type 2 diabetes, and obesity and influenced by sex: the ADDITION-PRO study. *Diabetes* **64**, 2513–2525 (2015).
47. Nauck, M. A. *et al.* Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in

patients with type-2 diabetes mellitus. *J. Clin. Invest.* **91**, 301–307 (1993).
48. Nauck, M. A. *et al.* Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7–36 amide) in type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* **36**, 741–744 (1993).
49. Larsen, J., Hylleberg, B., Ng, K. & Damsbo, P. Glucagon-like peptide-1 infusion must be maintained for 24 h/day to obtain acceptable glycemia in type 2 diabetic patients who are poorly controlled on sulphonylurea treatment. *Diabetes Care* **24**, 1416–1421 (2001).
50. Rachman, J., Barrow, B. A., Levy, J. C. & Turner, R. C. Near-normalisation of diurnal glucose concentrations by continuous administration of glucagon-like peptide-1 (GLP-1) in subjects with NIDDM. *Diabetologia* **40**, 205–211 (1997).
51. Zander, M., Madsbad, S., Madsen, J. L. & Holst, J. J. 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* **359**, 824–830 (2002).
Key proof-of-principal study that shows that continuous subcutaneous infusion of GLP-1 improves glycaemic control in patients with T2DM.
52. Deacon, C. F., Nauck, M. A., Meier, J., Hucking, K. & Holst, J. J. Degradation of endogenous and exogenous gastric inhibitory polypeptide in healthy and in type 2 diabetic subjects as revealed using a new assay for the intact peptide. *J. Clin. Endocrinol. Metab.* **85**, 3575–3581 (2000).
53. Mentlein, R., Gallwitz, B. & Schmidt, W. E. Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7–36)amide, peptide histidine methionine and is responsible for their degradation in human serum. *Eur. J. Biochem.* **214**, 829–835 (1993).
54. Hupe-Sodmann, K. *et al.* Endoproteolysis of glucagon-like peptide (GLP)-1 (7–36) amide by ectopeptidases in RINm5F cells. *Peptides* **18**, 625–632 (1997).
55. Hupe-Sodmann, K. *et al.* Characterisation of the processing by human neutral endopeptidase 24.11 of GLP-1(7–36) amide and comparison of the substrate specificity of the enzyme for other glucagon-like peptides. *Regul. Pept.* **58**, 149–156 (1995).
56. Mentlein, R. Mechanisms underlying the rapid degradation and elimination of the incretin hormones GLP-1 and GIP. *Best Pract. Res. Clin. Endocrinol. Metab.* **23**, 443–452 (2009).
57. Idorn, T. *et al.* Elimination and degradation of glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide in patients with end-stage renal disease. *J. Clin. Endocrinol. Metab.* **99**, 2457–2466 (2014).
58. Mulvihill, E. E. & Drucker, D. J. Pharmacology, physiology, and mechanisms of action of dipeptidyl peptidase-4 inhibitors. *Endocr. Rev.* **35**, 992–1019 (2014).
59. Tella, S. H. & Rendell, M. S. Glucagon-like polypeptide agonists in type 2 diabetes mellitus: efficacy and tolerability, a balance. *Ther. Adv. Endocrinol. Metab.* **6**, 109–134 (2015).
60. Meier, J. J. GLP-1 receptor agonists for individualized treatment of type 2 diabetes mellitus. *Nat. Rev. Endocrinol.* **8**, 728–742 (2012).
Early, in-depth review article that evaluates the similarities and differences between short-acting and long-acting GLP-1R agonists in terms of glucose-lowering efficacy and adverse effects, and discusses the possibilities for utilizing these agents for individualized treatment of T2DM.
61. Novo Nordisk. Novo nordisk files for regulatory approval of once-weekly semaglutide with the fda for the treatment of type 2 diabetes. *Novo Nordisk* http://press.novonordisk-us.com/2016-12-05-Novo-Nordisk-Files-for-Regulatory-Approval-of-Once-Weekly-Semaglutide-with-the-FDA-for-the-Treatment-of-Type-2-Diabetes (2016).
62. Davies, M., Chatterjee, S. & Khunti, K. The treatment of type 2 diabetes in the presence of renal impairment: what we should know about newer therapies. *Clin. Pharmacol.* **8**, 61–81 (2016).
63. Scheen, A. J. Pharmacokinetics and clinical use of incretin-based therapies in patients with chronic kidney disease and type 2 diabetes. *Clin. Pharmacokinet.* **54**, 1–21 (2015).
64. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT01394341 (2013).
65. Marso, S. P. *et al.* Liraglutide and cardiovascular outcomes in type 2 diabetes. *N. Engl. J. Med.* **375**, 311–322 (2016).
First cardiovascular safety trial to demonstrate that a GLP-1R agonist reduces MACE and microvascular (including renal) events in patients with T2DM and high cardiovascular risk.
66. Mann, J. F. Liraglutide and renal outcomes in type 2 diabetes: results of the LEADER trial. Oral Abstract Session. Kidney Week, November 15–20 https://www.asn-online.org/education/kidneyweek/archives/ (2016).
67. Shyangdan, D. S. *et al.* Glucagon-like peptide analogues for type 2 diabetes mellitus. *Cochrane Database Syst. Rev.* **5**, CD006423 (2011).
68. Buse, J. B. *et al.* Liraglutide once a day versus exenatide twice a day for type 2 diabetes: a 26-week randomised, parallel-group, multinational, open-label trial (LEAD-6). *Lancet* **374**, 39–47 (2009).
69. Drucker, D. J. *et al.* Exenatide once weekly versus twice daily for the treatment of type 2 diabetes: a randomised, open-label, non-inferiority study. *Lancet* **372**, 1240–1250 (2008).
70. Meier, J. J. *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* **38**, 1263–1273 (2015).
71. Kramer, C. K., Zinman, B., Choi, H., Connelly, P. W. & Retnakaran, R. The impact of chronic liraglutide therapy on glucagon secretion in type 2 diabetes: insight from the LIBRA trial. *J. Clin. Endocrinol. Metab.* **100**, 3702–3709 (2015).
72. Smits, M. M. *et al.* Effect of 3 years of treatment with exenatide on postprandial glucagon levels. *Diabetes Care* **39**, e42–e43 (2016).
73. Deacon, C. F. & Lebovitz, H. E. Comparative review of dipeptidyl peptidase-4 inhibitors and sulphonylureas. *Diabetes Obes. Metab.* **18**, 333–347 (2016).
74. Deacon, C. F. Dipeptidyl peptidase-4 inhibitors in the treatment of type 2 diabetes: a comparative review. *Diabetes Obes. Metab.* **13**, 7–18 (2011).
75. Bergman, A. J. *et al.* Effect of renal insufficiency on the pharmacokinetics of sitagliptin, a dipeptidyl peptidase-4 inhibitor. *Diabetes Care* **30**, 1862–1864 (2007).
76. Bailey, C. J. in *International Textbook of Diabetes Mellitus* 4th edn Ch 47 (eds DeFronzo, R.A., Ferrannini, E, Alberti, KG, Zimmet, P) (John Wiley & Sons Ltd., 2015).
77. Omar, B. & Ahren, B. Pleiotropic mechanisms for the glucose-lowering action of DPP-4 inhibitors. *Diabetes* **63**, 2196–2202 (2014).
78. Craddy, P., Palin, H. J. & Johnson, K. I. Comparative effectiveness of dipeptidylpeptidase-4 inhibitors in type 2 diabetes: a systematic review and mixed treatment comparison. *Diabetes Ther.* **5**, 1–41 (2014).
79. Esposito, K. *et al.* A nomogram to estimate the HbA1c response to different DPP-4 inhibitors in type 2 diabetes: a systematic review and meta-analysis of 98 trials with 24 163 patients. *BMJ Open* **5**, e005892 (2015).
80. Palmer, B. F. & Clegg, D. J. Physiology and pathophysiology of potassium homeostasis. *Adv. Physiol. Educ.* **40**, 480–490 (2016).
81. Hoorn, E. J. & Zietse, R. Gut-kidney kaliuretic signaling: looking forward to feeding. *Kidney Int.* **88**, 1230–1232 (2015).
82. Michell, A. R., Debnam, E. S. & Unwin, R. J. Regulation of renal function by the gastrointestinal tract: potential role of gut-derived peptides and hormones. *Annu. Rev. Physiol.* **70**, 379–403 (2008).
83. Thomas, L. & Kumar, R. Control of renal solute excretion by enteric signals and mediators. *J. Am. Soc. Nephrol.* **19**, 207–212 (2008).
84. Carey, R. M. Evidence for a splanchnic sodium input monitor regulating renal sodium excretion in man. Lack of dependence upon aldosterone. *Circ. Res.* **43**, 19–23 (1978).
85. Preston, R. A. *et al.* Sodium challenge does not support an acute gastrointestinal-renal natriuretic signaling axis in humans. *Kidney Int.* **82**, 1313–1320 (2012).
86. Singer, D. R. *et al.* Contrasting endocrine responses to acute oral compared with intravenous sodium loading in normal humans. *Am. J. Physiol.* **274**, F111–F119 (1998).
Clinical study that evaluated the endocrine mechanisms involved in accelerated natriuresis after oral compared with intravenous sodium loading in healthy individuals.
87. Yang, J., Jose, P. A. & Zeng, C. Gastrointestinal-renal axis: role in the regulation of blood pressure. *J. Am. Heart Assoc.* **6**, e005536 (2017).
88. Jose, P. A., Felder, R. A., Yang, Z., Zeng, C. & Eisner, G. M. Gastrorenal axis. *Hypertension* **67**, 1056–1063 (2016).
89. Bankir, L., Roussel, R. & Bouby, N. Protein- and diabetes-induced glomerular hyperfiltration: role of glucagon, vasopressin, and urea. *Am. J. Physiol. Renal Physiol.* **309**, F2–F23 (2015).
90. Tonneijck, L. *et al.* Glomerular hyperfiltration in diabetes: mechanisms, clinical significance, and treatment. *J. Am. Soc. Nephrol.* **28**, 1023–1039 (2017).
In-depth review of the hitherto proposed mechanisms involved in diabetes-related glomerular hyperfiltration, its clinical significance and available and emerging interventions that might attenuate this renal haemodynamic abnormality.
91. Kutina, A. V., Golosova, D. V., Marina, A. S., Shakhmatova, E. I. & Natochin, Y. V. Role of vasopressin in the regulation of renal sodium excretion: interaction with glucagon-like peptide-1. *J Neuroendocrinol.* http://dx.doi.org/10.1111/jne.12367 (2016).
92. Marina, A. S., Kutina, A. V., Shakhmatoba, E. I. & Natochin, Y. V. Involvement of glucagon-like peptide-1 in the regulation of selective excretion of sodium or chloride ions by the kidneys. *Bull. Exp. Biol. Med.* **162**, 436–440 (2017).
93. Marina, A. S., Kutina, A. V., Shakhmatova, E. I., Balbotkina, E. V. & Natochin, Y. V. Stimulation of glucagon-like peptide-1 secretion by water loading in human. *Dokl. Biol. Sci.* **459**, 323–325 (2014).
94. McKay, N. J., Galante, D. L. & Daniels, D. Endogenous glucagon-like peptide-1 reduces drinking behavior and is differentially engaged by water and food intakes in rats. *J. Neurosci.* **34**, 16417–16423 (2014).
95. Petersen, B., Christiansen, J. & Holst, J. J. A glucose-dependent mechanism in jejunum inhibits gastric acid secretion: a response mediated through enteroglucagon? *Scand. J. Gastroenterol.* **20**, 193–197 (1985).
96. Navarro, M. *et al.* Colocalization of glucagon-like peptide-1 (GLP-1) receptors, glucose transporter GLUT-2, and glucokinase mRNAs in rat hypothalamic cells: evidence for a role of GLP-1 receptor agonists as an inhibitory signal for food and water intake. *J. Neurochem.* **67**, 1982–1991 (1996).
97. Tang-Christensen, M. *et al.* Central administration of GLP-1-(7–36) amide inhibits food and water intake in rats. *Am. J. Physiol.* **271**, R848–R856 (1996).
98. Gutzwiller, J. P. *et al.* Glucagon-like peptide-1 is involved in sodium and water homeostasis in humans. *Digestion* **73**, 142–150 (2006).
99. McKay, N. J., Kanoski, S. E., Hayes, M. R. & Daniels, D. Glucagon-like peptide-1 receptor agonists suppress water intake independent of effects on food intake. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **301**, R1755–R1764 (2011).
100. McKay, N. J. & Daniels, D. Glucagon-like peptide-1 receptor agonist administration suppresses both water and saline intake in rats. *J. Neuroendocrinol.* **25**, 929–938 (2013).
101. Crajoinas, R. O. *et al.* Mechanisms mediating the diuretic and natriuretic actions of the incretin hormone glucagon-like peptide-1. *Am. J. Physiol. Renal Physiol.* **301**, F355–F363 (2011).
102. Fujita, H. *et al.* The protective roles of GLP-1R signaling in diabetic nephropathy: possible mechanism and therapeutic potential. *Kidney Int.* **85**, 579–589 (2014).
103. Jensen, E. P. *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.* **308**, F867–F877 (2015).
104. Kodera, R. *et al.* Glucagon-like peptide-1 receptor agonist ameliorates renal injury through its anti-inflammatory action without lowering blood glucose level in a rat model of type 1 diabetes. *Diabetologia* **54**, 965–978 (2011).
105. Korner, M., Stockli, M., Waser, B. & Reubi, J. C. GLP-1 receptor expression in human tumors and human normal tissues: potential for *in vivo* targeting. *J. Nucl. Med.* **48**, 736–743 (2007).
106. Pyke, C. *et al.* GLP-1 receptor localization in monkey and human tissue: novel distribution revealed with extensively validated monoclonal antibody. *Endocrinology* **155**, 1280–1290 (2014).

Study that gives important insight into the molecular mode of action of GLP-1 by identifying the cellular localization of GLP-1R in monkey and human tissue using the most extensively validated monoclonal antibody to date.

107. Schlatter, P., Beglinger, C., Drewe, J. & Gutmann, H. Glucagon-like peptide 1 receptor expression in primary porcine proximal tubular cells. *Regul. Pept.* **141**, 120–128 (2007).
108. Kirino, Y. *et al.* Interrelationship of dipeptidyl peptidase IV (DPP4) with the development of diabetes, dyslipidaemia and nephropathy: a streptozotocin-induced model using wild-type and DPP4-deficient rats. *J. Endocrinol.* **200**, 53–61 (2009).
109. Skov, J. *et al.* Glucagon-like peptide-1 (GLP-1): effect on kidney hemodynamics and renin-angiotensin-aldosterone system in healthy men. *J. Clin. Endocrinol. Metab.* **98**, E664–E671 (2013).
110. Gutzwiller, J. P. *et al.* Glucagon-like peptide 1 induces natriuresis in healthy subjects and in insulin-resistant obese men. *J. Clin. Endocrinol. Metab.* **89**, 3055–3061 (2004).
Clinical study demonstrating that acute GLP-1 infusion reduces glomerular hyperfiltration and produces an increase in urinary sodium excretion and a decrease in urinary hydrogen excretion in insulin-resistant obese men.
111. Farah, L. X. *et al.* The physiological role of glucagon-like peptide-1 in the regulation of renal function. *Am. J. Physiol. Renal Physiol.* **310**, F123–F127 (2016).
112. Muskiet, M. H. *et al.* Acute renal haemodynamic effects of glucagon-like peptide-1 receptor agonist exenatide in healthy overweight men. *Diabetes Obes. Metab.* **18**, 178–185 (2016).
113. Tonneijck, L. *et al.* Acute renal effects of the GLP-1 receptor agonist exenatide in overweight type 2 diabetes patients: a randomised, double-blind, placebo-controlled trial. *Diabetologia* **59**, 1412–1421 (2016).
114. Skov, J. *et al.* Short-term effects of liraglutide on kidney function and vasoactive hormones in type 2 diabetes: a randomized clinical trial. *Diabetes Obes. Metab.* **18**, 581–589 (2016).
115. Lovshin, J. A. *et al.* Liraglutide promotes natriuresis but does not increase circulating levels of atrial natriuretic peptide in hypertensive subjects with type 2 diabetes. *Diabetes Care* **38**, 132–139 (2015).
116. Tonneijck, L. *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* **39**, 2042–2050 (2016).
117. Tonneijck, L. *et al.* Postprandial renal haemodynamic effect of lixisenatide versus once-daily insulin glulisine in type 2 diabetes patients on insulin-glargine: an 8-week, randomised, open-label trial. *Diabetes Obes. Metab.* http://dx.doi.org/10.1111/dom.12985 (2017).
118. Zhou, X. *et al.* Acute hemodynamic and renal effects of glucagon-like peptide 1 analog and dipeptidyl peptidase-4 inhibitor in rats. *Cardiovasc. Diabetol.* **14**, 29 (2015).
119. Takashima, S. *et al.* Stromal cell-derived factor-1 is upregulated by dipeptidyl peptidase-4 inhibition and has protective roles in progressive diabetic nephropathy. *Kidney Int.* **90**, 783–796 (2016).
120. Lovshin, J. A. *et al.* Dipeptidyl peptidase-4 inhibition stimulates distal tubular natriuresis and increases in circulating SDF-1alpha1-67 in patients with type 2 diabetes. *Diabetes Care* **40**, 1073–1081 (2017).
Clinical study suggesting that DPP-4 inhibition blocks distal, rather than proximal, sodium reabsorption after prolonged therapy in patients with T2DM, potentially as a result of decreased inactivation of SDF1α rather than a mechanism involving GLP-1.
121. Girardi, A. C., Fukuda, L. E., Rossoni, L. V., Malnic, G. & Reboucas, N. A. Dipeptidyl peptidase IV inhibition downregulates Na^+-H^+ exchanger NHE3 in rat renal proximal tubule. *Am. J. Physiol. Renal Physiol.* **294**, F414–F422 (2008).
122. Girardi, A. C., Knauf, F., Demuth, H. U. & Aronson, P. S. Role of dipeptidyl peptidase IV in regulating activity of Na^+/H^+ exchanger isoform NHE3 in proximal tubule cells. *Am. J. Physiol. Cell Physiol.* **287**, C1238–C1245 (2004).
123. Carraro-Lacroix, L. R., Malnic, G. & Girardi, A. C. Regulation of Na^+/H^+ exchanger NHE3 by glucagon-like peptide 1 receptor agonist exendin-4 in renal proximal tubule cells. *Am. J. Physiol. Renal Physiol.* **297**, F1647–F1655 (2009).
124. Moreno, C., Mistry, M. & Roman, R. J. Renal effects of glucagon-like peptide in rats. *Eur. J. Pharmacol.* **434**, 163–167 (2002).
125. McDonough, A. A., Leong, P. K. & Yang, L. E. Mechanisms of pressure natriuresis: how blood pressure regulates renal sodium transport. *Ann. NY Acad. Sci.* **986**, 669–677 (2003).
126. Rieg, T. *et al.* Natriuretic effect by exendin-4, but not the DPP-4 inhibitor alogliptin, is mediated via the GLP-1 receptor and preserved in obese type 2 diabetic mice. *Am. J. Physiol. Renal Physiol.* **303**, F963–F971 (2012).
127. Tonneijck, L., Muskiet, M. H., Smits, M. M., van Raalte, D. H. & Diamant, M. Combining incretin-based drugs and RAAS inhibitors: more cons than pros? *Lancet Diabetes Endocrinol.* **2**, 684–685 (2014).
128. Asmar, A. *et al.* Renal extraction and acute effects of glucagon-like peptide-1 on central and renal hemodynamics in healthy men. *Am. J. Physiol. Endocrinol. Metab.* **308**, E641–E649 (2015).
129. Asmar, A. *et al.* Glucagon-like peptide-1 does not have acute effects on central or renal hemodynamics in patients with type 2 diabetes without nephropathy. *Am. J. Physiol. Endocrinol. Metab.* **310**, E744–E753 (2016).
130. Kim, M. *et al.* GLP-1 receptor activation and Epac2 link atrial natriuretic peptide secretion to control of blood pressure. *Nat. Med.* **19**, 567–575 (2013).
131. Skov, J., Holst, J. J., Gotze, J. P., Frokiaer, J. & Christiansen, J. S. Glucagon-like peptide-1: effect on pro-atrial natriuretic peptide in healthy males. *Endocr. Connect.* **3**, 11–16 (2014).
132. Marina, A. S., Kutina, A. V. & Natochin, Y. V. Exenatide stimulates solute-free water clearance by the rat kidney in hyperhydration. *Dokl. Biol. Sci.* **437**, 85–87 (2011).
133. Kutina, A. V., Marina, A. S., Shakhmatova, E. I. & Natochin, Y. V. Physiological mechanisms for the increase in renal solute-free water clearance by a glucagon-like peptide-1 mimetic. *Clin. Exp. Pharmacol. Physiol.* **40**, 510–517 (2013).
134. Shakhmatova, E. I. *et al.* Exenatide stimulated solute-free water excretion by human kidney [Russian]. *Ross. Fiziol. Zh. Im. I. M. Sechenova* **98**, 1021–1029 (2012).
135. Thomson, S. C., Kashkouli, A. & Singh, P. Glucagon-like peptide-1 receptor stimulation increases GFR and suppresses proximal reabsorption in the rat. *Am. J. Physiol. Renal Physiol.* **304**, F137–F144 (2013).
136. Thomson, S. C. *et al.* Temporal adjustment of the juxtaglomerular apparatus during sustained inhibition of proximal reabsorption. *J. Clin. Invest.* **104**, 1149–1158 (1999).
137. von Scholten, B. J., Hansen, T. W., Goetze, J. P., Persson, F. & Rossing, P. Glucagon-like peptide 1 receptor agonist (GLP-1 RA): long-term effect on kidney function in patients with type 2 diabetes. *J. Diabetes Complications* **29**, 670–674 (2015).
138. von Scholten, B. J., Lajer, M., Goetze, J. P., Persson, F. & Rossing, P. Time course and mechanisms of the anti-hypertensive and renal effects of liraglutide treatment. *Diabet. Med.* **32**, 343–352 (2015).
139. von Scholten, B. J. *et al.* The effect of liraglutide on renal function: a randomized clinical trial. *Diabetes Obes. Metab.* **19**, 239–247 (2017).
Placebo-controlled cross-over trial in patients with T2DM and albuminuria that shows that liraglutide therapy reduces 24-h urinary albumin excretion by lowering blood pressure, rather than by improving HbA_{1c} levels and body weight.
140. Tonneijck, L., Smits, M. M., van Raalte, D. H. & Muskiet, M. H. Incretin-based drugs and renoprotection-is hyperfiltration key? *Kidney Int.* **87**, 660–661 (2015).
141. Cherney, D. Z. *et al.* Renal hemodynamic effect of sodium-glucose cotransporter 2 inhibition in patients with type 1 diabetes mellitus. *Circulation* **129**, 587–597 (2014).
First description in humans of the ability of SGLT2 inhibitors to attenuate glomerular hyperfiltration, likely by affecting tubuloglomerular feedback mechanisms.
142. Park, C. W. *et al.* Long-term treatment of glucagon-like peptide-1 analog exendin-4 ameliorates diabetic nephropathy through improving metabolic anomalies in db/db mice. *J. Am. Soc. Nephrol.* **18**, 1227–1238 (2007).
143. Arjona Ferreira, J. C. *et al.* Efficacy and safety of sitagliptin versus glipizide in patients with type 2 diabetes and moderate-to-severe chronic renal insufficiency. *Diabetes Care* **36**, 1067–1073 (2013).
144. Davies, M. J. *et al.* Efficacy and safety of liraglutide versus placebo as add-on to glucose-lowering therapy in patients with type 2 diabetes and moderate renal impairment (LIRA-RENAL): a randomized clinical trial. *Diabetes Care* **39**, 222–230 (2016).
145. Groop, P. H. *et al.* Linagliptin treatment in subjects with type 2 diabetes with and without mild-to-moderate renal impairment. *Diabetes Obes. Metab.* **16**, 560–568 (2014).
146. McGill, J. B. *et al.* Long-term efficacy and safety of linagliptin in patients with type 2 diabetes and severe renal impairment: a 1-year, randomized, double-blind, placebo-controlled study. *Diabetes Care* **36**, 237–244 (2013).
147. Cooper, M. E. *et al.* Kidney disease end points in a pooled analysis of individual patient-level data from a large clinical trials program of the dipeptidyl peptidase 4 inhibitor linagliptin in type 2 diabetes. *Am. J. Kidney Dis.* **66**, 441–449 (2015).
148. Tuttle, K. R., Heilmann, C., Hoogwerf, B. J., Brown, C. & Anderson, P. W. Effects of exenatide on kidney function, adverse events, and clinical end points of kidney disease in type 2 diabetes. *Am. J. Kidney Dis.* **62**, 396–398 (2013).
149. Tuttle, K. R. *et al.* Effects of once-weekly dulaglutide on kidney function in patients with type 2 diabetes in phase II and III clinical trials. *Diabetes Obes. Metab.* **19**, 436–441 (2017).
Analysis of pooled phase II and phase III RCTs that describes the albuminuria-lowering effect of dulaglutide compared to insulin glargine and other active comparators beyond glycaemic control.
150. Ott, C. *et al.* Effects of linagliptin on renal endothelial function in patients with type 2 diabetes: a randomised clinical trial. *Diabetologia* **59**, 2579–2587 (2016).
151. Gaede, P. *et al.* Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. *N. Engl. J. Med.* **348**, 383–393 (2003).
152. Oellgaard, J. *et al.* Intensified multifactorial intervention in type 2 diabetics with microalbuminuria leads to long-term renal benefits. *Kidney Int.* **91**, 982–988 (2017).
Key *post hoc* analysis of the landmark Steno-2 trial showing that intensified, multifactorial treatment slows progression of diabetic nephropathy and renal function loss in microalbuminuric patients with T2DM.
153. Ali, M. K. *et al.* Achievement of goals in U.S. diabetes care, 1999–2010. *N. Engl. J. Med.* **368**, 1613–1624 (2013).
154. Liu, S. C., Tu, Y. K., Chien, M. N. & Chien, K. L. Effect of antidiabetic agents added to metformin on glycaemic control, hypoglycaemia and weight change in patients with type 2 diabetes: a network meta-analysis. *Diabetes Obes. Metab.* **14**, 810–820 (2012).
155. Sun, F. *et al.* Effects of glucagon-like peptide-1 receptor agonists on weight loss in patients with type 2 diabetes: a systematic review and network meta-analysis. *J. Diabetes Res.* **2015**, 157201 (2015).
156. Pi-Sunyer, X. *et al.* A randomized, controlled trial of 3.0 mg of liraglutide in weight management. *N. Engl. J. Med.* **373**, 11–22 (2015).
157. European Medicines Agency. Saxenda. *EMA* http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/003780/human_med_001855.jsp&mid=WC0b01ac058001d124 (2017).
158. Bunck, M. C. *et al.* Exenatide affects circulating cardiovascular risk biomarkers independently of changes in body composition. *Diabetes Care* **33**, 1734–1737 (2010).
159. Jendle, J. *et al.* Weight loss with liraglutide, a once-daily human glucagon-like peptide-1 analogue for type 2 diabetes treatment as monotherapy or added to metformin, is primarily as a result of a reduction in fat tissue. *Diabetes Obes. Metab.* **11**, 1163–1172 (2009).
160. Edwards, C. M., Todd, J. F., Ghatei, M. A. & Bloom, S. R. Subcutaneous glucagon-like peptide-1 (7–36) amide is insulinotropic and can cause hypoglycaemia in fasted healthy subjects. *Clin. Sci. (Lond.)* **95**, 719–724 (1998).
161. Halbirk, M. *et al.* Cardiovascular and metabolic effects of 48-h glucagon-like peptide-1 infusion in

compensated chronic patients with heart failure. *Am. J. Physiol. Heart Circ. Physiol.* **298**, H1096–H1102 (2010).
162. Sun, F. *et al.* Impact of GLP-1 receptor agonists on blood pressure, heart rate and hypertension among patients with type 2 diabetes: a systematic review and network meta-analysis. *Diabetes Res. Clin. Pract.* **110**, 26–37 (2015).
163. Zhang, X. & Zhao, Q. Effects of dipeptidyl peptidase-4 inhibitors on blood pressure in patients with type 2 diabetes: a systematic review and meta-analysis. *J. Hypertens.* **34**, 167–175 (2016).
164. Davies, M. J. *et al.* Efficacy of liraglutide for weight loss among patients with type 2 diabetes: the SCALE diabetes randomized clinical trial. *JAMA* **314**, 687–699 (2015).
Study that establishes the efficacy and safety of liraglutide in patients with T2DM and moderate renal impairment, but shows a lack of effect of acute and prolonged therapy with this GLP-1R agonist on eGFR trajectory compared to placebo.
165. Sun, F. *et al.* Effect of glucagon-like peptide-1 receptor agonists on lipid profiles among type 2 diabetes: a systematic review and network meta-analysis. *Clin. Ther.* **37**, 225–241.e8 (2015).
166. Monami, M., Lamanna, C., Desideri, C. M. & Mannucci, E. DPP-4 inhibitors and lipids: systematic review and meta-analysis. *Adv. Ther.* **29**, 14–25 (2012).
167. Drucker, D. J. The cardiovascular biology of glucagon-like peptide-1. *Cell Metab.* **24**, 15–30 (2016).
Authoritative review describing how GLP-1 and clinically approved GLP-1R agonists engage mechanisms that influence the risk of developing cardiovascular disease in diabetes and obesity.
168. Smits, M. M. *et al.* Twelve week liraglutide or sitagliptin does not affect hepatic fat in type 2 diabetes: a randomised placebo-controlled trial. *Diabetologia* **59**, 2588–2593 (2016).
169. Kooijman, S. *et al.* Central GLP-1 receptor signalling accelerates plasma clearance of triacylglycerol and glucose by activating brown adipose tissue in mice. *Diabetologia* **58**, 2637–2646 (2015).
170. Alter, M. L. *et al.* DPP-4 inhibition on top of angiotensin receptor blockade offers a new therapeutic approach for diabetic nephropathy. *Kidney Blood Press. Res.* **36**, 119–130 (2012).
171. Brosius, F. C. *et al.* Mouse models of diabetic nephropathy. *J. Am. Soc. Nephrol.* **20**, 2503–2512 (2009).
172. Nakagawa, T. *et al.* Diabetic endothelial nitric oxide synthase knockout mice develop advanced diabetic nephropathy. *J. Am. Soc. Nephrol.* **18**, 539–550 (2007).
173. Jung, E., Kim, J., Ho Kim, S., Kim, S. & Cho, M. H. Gemigliptin improves renal function and attenuates podocyte injury in mice with diabetic nephropathy. *Eur. J. Pharmacol.* **761**, 116–124 (2015).
174. Jung, G. S. *et al.* Renoprotective effect of gemigliptin, a dipeptidyl peptidase-4 inhibitor, in streptozotocin-induced type 1 diabetic mice. *Diabetes Metab. J.* **40**, 211–221 (2016).
175. Kodera, R. *et al.* Dipeptidyl peptidase-4 inhibitor ameliorates early renal injury through its anti-inflammatory action in a rat model of type 1 diabetes. *Biochem. Biophys. Res. Commun.* **443**, 828–833 (2014).
176. Liu, W. J. *et al.* Dipeptidyl peptidase IV inhibitor attenuates kidney injury in streptozotocin-induced diabetic rats. *J. Pharmacol. Exp. Ther.* **340**, 248–255 (2012).
177. Wang, Y. *et al.* Attenuation of renovascular damage in zucker diabetic fatty rat by NWT-03, an egg protein hydrolysate with ACE- and DPP4-inhibitory activity. *PLoS ONE* **7**, e46781 (2012).
178. Zhou, S. J. *et al.* Liraglutide ameliorates renal injury in streptozotocininduced diabetic rats by activating endothelial nitric oxide synthase activity via the downregulation of the nuclear factor-κB pathway. *Mol. Med. Rep.* **10**, 2587–2594 (2014).
179. Hendarto, H. *et al.* GLP-1 analog liraglutide protects against oxidative stress and albuminuria in streptozotocin-induced diabetic rats via protein kinase A-mediated inhibition of renal NAD(P)H oxidases. *Metabolism* **61**, 1422–1434 (2012).
180. Li, W. *et al.* Inhibition of the expression of TGF-beta1 and CTGF in human mesangial cells by exendin-4, a glucagon-like peptide-1 receptor agonist. *Cell. Physiol. Biochem.* **30**, 749–757 (2012).
181. Sourris, K. C. *et al.* Can targeting the incretin pathway dampen RAGE-mediated events in diabetic nephropathy? *Curr. Drug Targets* **17**, 1252–1264 (2016).
182. Ishibashi, Y. *et al.* Glucagon-like peptide-1 inhibits angiotensin II-induced mesangial cell damage via protein kinase A. *Microvasc. Res.* **84**, 395–398 (2012).
183. Mima, A. *et al.* Protective effects of GLP-1 on glomerular endothelium and its inhibition by PKCβ activation in diabetes. *Diabetes* **61**, 2967–2979 (2012).
184. Nistala, R. *et al.* DPP4 inhibition attenuates filtration barrier injury and oxidant stress in the zucker obese rat. *Obesity (Silver Spring)* **22**, 2172–2179 (2014).
185. Kanasaki, K. *et al.* Linagliptin-mediated DPP-4 inhibition ameliorates kidney fibrosis in streptozotocin-induced diabetic mice by inhibiting endothelial-to-mesenchymal transition in a therapeutic regimen. *Diabetes* **63**, 2120–2131 (2014).
Preclinical study in mice with streptozotocin-induced diabetes that showed glucose-independent amelioration of kidney fibrosis with the DPP-4 inhibitor linagliptin.
186. Shi, S. *et al.* Interactions of DPP-4 and integrin beta1 influences endothelial-to-mesenchymal transition. *Kidney Int.* **88**, 479–489 (2015).
187. Panchapakesan, U. & Pollock, C. A. DPP-4 inhibitors-renoprotection in diabetic nephropathy? *Diabetes* **63**, 1829–1830 (2014).
188. Zeisberg, M. & Zeisberg, E. M. Evidence for antifibrotic incretin-independent effects of the DPP-4 inhibitor linagliptin. *Kidney Int.* **88**, 429–431 (2015).
189. Derosa, G. *et al.* Exenatide versus glibenclamide in patients with diabetes. *Diabetes Technol. Ther.* **12**, 233–240 (2010).
190. Rizzo, M. *et al.* Liraglutide reduces oxidative stress and restores heme oxygenase-1 and ghrelin levels in patients with type 2 diabetes: a prospective pilot study. *J. Clin. Endocrinol. Metab.* **100**, 603–606 (2015).
191. Zhang, H., Zhang, X., Hu, C. & Lu, W. Exenatide reduces urinary transforming growth factor-beta1 and type IV collagen excretion in patients with type 2 diabetes and microalbuminuria. *Kidney Blood Press. Res.* **35**, 483–488 (2012).
192. Imamura, S., Hirai, K. & Hirai, A. The glucagon-like peptide-1 receptor agonist, liraglutide, attenuates the progression of overt diabetic nephropathy in type 2 diabetic patients. *Tohoku J. Exp. Med.* **231**, 57–61 (2013).
193. Zavattaro, M. *et al.* One-year treatment with liraglutide improved renal function in patients with type 2 diabetes: a pilot prospective study. *Endocrine* **50**, 620–626 (2015).
194. Pawaskar, M., Tuttle, K. R., Li, Q., Best, J. H. & Anderson, P. W. Observational study of kidney function and albuminuria in patients with type 2 diabetes treated with exenatide BID versus insulin glargine. *Ann. Pharmacother.* **48**, 571–576 (2014).
195. Tuttle, K. R. *et al.* Dulaglutide vs. glargine, both combined with lispro, mitigated eGFR decline in people with type 2 diabetes and moderate-to-severe chronic kidney disease (AWARD-7). Presented at ADA's 77th Scientific Sessions, San Diego, California, USA. Poster number: 142-LB (2017).
196. Zheng, T. *et al.* Increased plasma dipeptidyl peptidase-4 activities are associated with high prevalence of diabetic nephropathy in Chinese patients with newly diagnosed type 2 diabetes: a cross-sectional study. *Diab. Vasc. Dis. Res.* **13**, 127–136 (2016).
197. Duvnjak, L., Perkovic, M. N. & Blaslov, K. Dipeptidyl peptidase-4 activity is associated with urine albumin excretion in type 1 diabetes. *J. Diabetes Complications* **31**, 218–222 (2017).
198. Zheng, T., Baskota, A., Gao, Y., Tian, H. & Yang, F. Increased plasma dipeptidyl peptidase 4 activities predict new-onset microalbuminuria in association with its proinflammatory effects in Chinese without diabetes: a four-year prospective study. *Nephrol. Dial. Transplant.* **30**, 460–466 (2015).
199. Sun, A. L. *et al.* Dipeptidyl peptidase-IV is a potential molecular biomarker in diabetic kidney disease. *Diab. Vasc. Dis. Res.* **9**, 301–308 (2012).
200. Mitic, B., Lazarevic, G., Vlahovic, P., Rajic, M. & Stefanovic, V. Diagnostic value of the aminopeptidase N, N-acetyl-beta-D-glucosaminidase and dipeptidylpeptidase IV in evaluating tubular dysfunction in patients with glomerulopathies. *Ren. Fail.* **30**, 896–903 (2008).
201. Shi, S., Koya, D. & Kanasaki, K. Dipeptidyl peptidase-4 and kidney fibrosis in diabetes. *Fibrogen. Tissue Repair* **9**, 1 (2016).
202. Sakata, K. *et al.* Efficacy of alogliptin, a dipeptidyl peptidase-4 inhibitor, on glucose parameters, the activity of the advanced glycation end product (AGE) - receptor for AGE (RAGE) axis and albuminuria in Japanese type 2 diabetes. *Diabetes Metab. Res. Rev.* **29**, 624–630 (2013).
203. Hattori, S. Sitagliptin reduces albuminuria in patients with type 2 diabetes. *Endocr. J.* **58**, 69–73 (2011).
204. Iazzetta, N. *et al.* Nephroprotection with saxagliptin. *G. Ital. Nefrol.* **32**, gin/32.6.11 (2015).
205. Kim, Y. G. *et al.* Renal protective effect of dpp-4 inhibitors in type 2 diabetes mellitus patients: a cohort study. *J. Diabetes Res.* **2016**, 1423191 (2016).
206. Groop, P. H. *et al.* Linagliptin lowers albuminuria on top of recommended standard treatment in patients with type 2 diabetes and renal dysfunction. *Diabetes Care* **36**, 3460–3468 (2013).
207. Groop, P. H. *et al.* Linagliptin and its effects on hyperglycaemia and albuminuria in patients with type 2 diabetes and renal dysfunction: the randomized MARLINA-T2D trial. *Diabetes Obes. Metab.* http://dx.doi.org/10.1111/dom.13041 (2017).
This sufficiently powered clinical study did not find a beneficial effect of linagliptin versus placebo on albuminuria in patients with T2DM, although exploratory analyses indicated more responders and fewer non-responders in terms of UACR in the linagliptin group.
208. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT01897532 (2017).
209. Mori, H., Okada, Y., Arao, T. & Tanaka, Y. Sitagliptin improves albuminuria in patients with type 2 diabetes mellitus. *J. Diabetes Investig.* **5**, 313–319 (2014).
210. Bergenstal, R. M. *et al.* Efficacy and safety of exenatide once weekly versus sitagliptin or pioglitazone as an adjunct to metformin for treatment of type 2 diabetes (DURATION-2): a randomised trial. *Lancet* **376**, 431–439 (2010).
211. Food and Drug Administration. Guidance for industry: diabetes mellitus — evaluating cardiovascular risk in new antidiabetic therapies to treat type 2 diabetes. *FDA* http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm071627.pdf (2008).
212. Pfeffer, M. A., Claggett, B. & Probstfield, J. L. Lixisenatide in type 2 diabetes and acute coronary syndrome. *N. Engl. J. Med.* **374**, 1095–1096 (2016).
The first cardiovascular safety trial of a GLP-1R agonist, demonstrating that lixisenatide does not augment MACE in patients with T2DM who had a recent acute coronary event.
213. Marso, S. P. *et al.* Semaglutide and cardiovascular outcomes in patients with type 2 diabetes. *N. Engl. J. Med.* **375**, 1834–1844 (2016).
Second cardiovascular safety trial to demonstrate that a GLP-1R agonist reduces MACE and microvascular (including renal) events in patients with T2DM and high cardiovascular risk.
214. Muskiet, M. H., Tonneijck, L., van Bommel, E. J., Smits, M. M. & van Raalte, D. H. Renoprotection in LEADER and EMPA-REG OUTCOME. *Lancet Diabetes Endocrinol.* **4**, 812–814 (2016).
215. Scirica, B. M. *et al.* Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. *N. Engl. J. Med.* **369**, 1317–1326 (2013).
First cardiovascular safety trial of a DPP-4 inhibitor in patients with T2DM and high cardiovascular risk, showing that saxagliptin does not augment MACE but increases hospitalization for heart failure.
216. White, W. B. *et al.* Alogliptin after acute coronary syndrome in patients with type 2 diabetes. *N. Engl. J. Med.* **369**, 1327–1335 (2013).
Cardiovascular safety trial demonstrating that the DPP-4 inhibitor alogliptin does not increase MACE in patients with T2DM who had a recent acute coronary event.
217. Green, J. B. *et al.* Effect of sitagliptin on cardiovascular outcomes in type 2 diabetes. *N. Engl. J. Med.* **373**, 232–242 (2015).
Cardiovascular safety trial demonstrating that the DPP-4 inhibitor sitagliptin does not increase MACE or hospitalization for heart failure in patients with T2DM and high cardiovascular risk.
218. Kongwatcharapong, J., Dilokthornsakul, P., Nathisuwan, S., Phrommintikul, A. & Chaiyakunapruk, N. Effect of dipeptidyl peptidase-4 inhibitors on heart failure: a meta-analysis of randomized clinical trials. *Int. J. Cardiol.* **211**, 88–95 (2016).

219. Scirica, B. M. *et al.* Heart failure, saxagliptin, and diabetes mellitus: observations from the SAVOR-TIMI 53 randomized trial. *Circulation* **130**, 1579–1588 (2014).
220. Muskiet, M. H. *et al.* Regarding article, "Heart failure, saxagliptin, and diabetes mellitus: observations from the SAVOR-TIMI 53 randomized trial". *Circulation* **132**, e120 (2015).
221. Scirica, B. M. *et al.* Response to letter regarding article, "Heart failure, saxagliptin and diabetes mellitus: observations from the SAVOR-TIMI 53 randomized trial". *Circulation* **132**, e121–e122 (2015).
222. White, W. B. *et al.* Angiotensin-converting enzyme inhibitor use and major cardiovascular outcomes in type 2 diabetes mellitus treated with the dipeptidyl peptidase 4 inhibitor alogliptin. *Hypertension* **68**, 606–613 (2016).
223. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT02130687 (2016).
224. Mosenzon, O. *et al.* Effect of saxagliptin on renal outcomes in the SAVOR-TIMI 53 trial. *Diabetes Care* **40**, 69–76 (2017).
Secondary analysis of renal end points in a cardiovascular safety trial of a DPP-4 inhibitor in patients with T2DM at high cardiovascular risk, showing that saxagliptin reduces albuminuria in a glucose-independent manner.
225. Cornel, J. H. *et al.* Effect of sitagliptin on kidney function and respective cardiovascular outcomes in type 2 diabetes: outcomes from TECOS. *Diabetes Care* **39**, 2304–2310 (2016).
226. Nauck, M. Incretin therapies: highlighting common features and differences in the modes of action of glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors. *Diabetes Obes. Metab.* **18**, 203–216 (2016).
227. Storgaard, H., Cold, F., Gluud, L. L., Vilsboll, T. & Knop, F. K. Glucagon-like peptide-1 agonists and risk of acute pancreatitis in patients with type 2 diabetes. *Diabetes Obes. Metab.* http://dx.doi.org/10.1111/dom.12885 (2017).
228. Abbas, A. S., Dehbi, H. M. & Ray, K. K. Cardiovascular and non-cardiovascular safety of dipeptidyl peptidase-4 inhibition: a meta-analysis of randomized controlled cardiovascular outcome trials. *Diabetes Obes. Metab.* **18**, 295–299 (2016).
229. Azoulay, L. *et al.* Incretin based drugs and the risk of pancreatic cancer: international multicentre cohort study. *BMJ* **352**, i581 (2016).
230. Shih, C. J. *et al.* Association between use of dipeptidyl peptidase-4 inhibitors and the risk of acute kidney injury: a nested case-control study. *Mayo Clin. Proc.* **91**, 867–872 (2016).
231. Pendergrass, M., Fenton, C., Haffner, S. M. & Chen, W. Exenatide and sitagliptin are not associated with increased risk of acute renal failure: a retrospective claims analysis. *Diabetes Obes. Metab.* **14**, 596–600 (2012).
232. Chang, M. W. *et al.* Sitagliptin protects rat kidneys from acute ischemia-reperfusion injury via upregulation of GLP-1 and GLP-1 receptors. *Acta Pharmacol. Sin.* **36**, 119–130 (2015).
233. Chen, Y. T. *et al.* Exendin-4 and sitagliptin protect kidney from ischemia-reperfusion injury through suppressing oxidative stress and inflammatory reaction. *J. Transl Med.* **11**, 270 (2013).
234. Glorie, L. L. *et al.* DPP4 inhibition improves functional outcome after renal ischemia-reperfusion injury. *Am. J. Physiol. Renal Physiol.* **303**, F681–F688 (2012).
235. Nuransoy, A. *et al.* Protective effect of sitagliptin against renal ischemia reperfusion injury in rats. *Ren. Fail.* **37**, 687–693 (2015).
236. Vaghasiya, J., Sheth, N., Bhalodia, Y. & Manek, R. Sitagliptin protects renal ischemia reperfusion induced renal damage in diabetes. *Regul. Pept.* **166**, 48–54 (2011).
237. Youssef, M. I., Mahmoud, A. A. & Abdelghany, R. H. A new combination of sitagliptin and furosemide protects against remote myocardial injury induced by renal ischemia/reperfusion in rats. *Biochem. Pharmacol.* **96**, 20–29 (2015).
238. Yang, H. *et al.* Exendin-4 ameliorates renal ischemia-reperfusion injury in the rat. *J. Surg. Res.* **185**, 825–832 (2013).
239. Chao, C. T., Wang, J., Wu, H. Y., Chien, K. L. & Hung, K. Y. Dipeptidyl peptidase 4 inhibitor use is associated with a lower risk of incident acute kidney injury in patients with diabetes. *Oncotarget* http://dx.doi.org/10.18632/oncotarget.18081 (2017).
240. Keller, J. *et al.* Effect of exenatide on cholecystokinin-induced gallbladder emptying in fasting healthy subjects. *Regul. Pept.* **179**, 77–83 (2012).
241. Smits, M. M. *et al.* Biliary effects of liraglutide and sitagliptin, a 12-week randomized placebo-controlled trial in type 2 diabetes patients. *Diabetes Obes. Metab.* **18**, 1217–1225 (2016).
242. [No authors listed.] Early worsening of diabetic retinopathy in the diabetes control and complications trial. *Arch. Ophthalmol.* **116**, 874–886 (1998).
243. Ipp, E., Genter, P. & Childress, K. Semaglutide and cardiovascular outcomes in patients with type 2 diabetes. *N. Engl. J. Med.* **376**, 890–891 (2017).
244. Vilsboll, T. Cardiovascular outcomes with semaglutide in subjects with type 2 diabetes mellitus (SUSTAIN 6). 77 th American Diabetes Association 2017 Scientific Sessions. June 9, San Diego, California. Presentation 1-AC-SY09 (2017).
245. Cherney, D. Z. I. *et al.* Effects of empagliflozin on the urinary albumin-to-creatinine ratio in patients with type 2 diabetes and established cardiovascular disease: an exploratory analysis from the EMPA-REG OUTCOME randomised, placebo-controlled trial. *Lancet Diabetes Endocrinol.* **5**, 610–621 (2017).
246. Muskiet, M. H. A., Heerspink, H. J. L. & van Raalte, D. H. SGLT2 inhibition: a new era in renoprotective medicine? *Lancet Diabetes Endocrinol.* **5**, 569–571 (2017).
247. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT01243424 (2017).
248. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT01794143 (2017).
249. Ferrannini, G. *et al.* Energy balance after sodium-glucose cotransporter 2 inhibition. *Diabetes Care* **38**, 1730–1735 (2015).
250. Frias, J. P. *et al.* Exenatide once weekly plus dapagliflozin once daily versus exenatide or dapagliflozin alone in patients with type 2 diabetes inadequately controlled with metformin monotherapy (DURATION-8): a 28 week, multicentre, double-blind, phase 3, randomised controlled trial. *Lancet Diabetes Endocrinol.* **4**, 1004–1016 (2016).
First clinical trial to investigate the efficacy and safety of combined SGLT2 inhibitor and GLP-1R agonist therapy in patients with T2DM, suggesting additive or synergistic effects on weight loss.
251. Nauck, M. A. & Meier, J. J. GLP-1 receptor agonists and SGLT2 inhibitors: a couple at last? *Lancet Diabetes Endocrinol.* **4**, 963–964 (2016).
252. Jackson, E. K., Kochanek, S. J. & Gillespie, D. G. Dipeptidyl peptidase IV regulates proliferation of preglomerular vascular smooth muscle and mesangial cells. *Hypertension* **60**, 757–764 (2012).
253. Kettmann, U., Humbel, B. & Holzhausen, H. J. Ultrastructural localization of dipeptidylpeptidase IV in the glomerulum of the rat kidney. *Acta Histochem.* **92**, 225–227 (1992).
254. Lee, J. W., Chou, C. L. & Knepper, M. A. Deep sequencing in microdissected renal tubules identifies nephron segment-specific transcriptomes. *J. Am. Soc. Nephrol.* **26**, 2669–2677 (2015).
255. European Medicines Agency. European public assessment reports. *EMA* http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/landing/epar_search.jsp&mid=WC0b01ac058001d124 (2017).
256. Nauck, M. A. *et al.* 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* **39**, 231–241 (2016).
257. Aaboe, K. *et al.* Twelve weeks treatment with the DPP-4 inhibitor, sitagliptin, prevents degradation of peptide YY and improves glucose and non-glucose induced insulin secretion in patients with type 2 diabetes mellitus. *Diabetes Obes. Metab.* **12**, 323–333 (2010).
258. Ballantyne, G. H. Peptide YY(1–36) and peptide YY(3–36): part i. distribution, release and actions. *Obes. Surg.* **16**, 651–658 (2006).
259. Nystrom, T., Gonon, A. T., Sjoholm, A. & Pernow, J. Glucagon-like peptide-1 relaxes rat conduit arteries via an endothelium-independent mechanism. *Regul. Pept.* **125**, 173–177 (2005).
260. Nystrom, T. *et al.* Effects of glucagon-like peptide-1 on endothelial function in type 2 diabetes patients with stable coronary artery disease. *Am. J. Physiol. Endocrinol. Metab.* **287**, E1209–E1215 (2004).
261. Lovshin, J. A. & Zinman, B. Blood pressure-lowering effects of incretin-based diabetes therapies. *Can. J. Diabetes* **38**, 364–371 (2014).
262. Girardi, A. C., Degray, B. C., Nagy, T., Biemesderfer, D. & Aronson, P. S. Association of Na^+-H^+ exchanger isoform NHE3 and dipeptidyl peptidase IV in the renal proximal tubule. *J. Biol. Chem.* **276**, 46671–46677 (2001).
263. Vanderheyden, M. *et al.* Dipeptidyl-peptidase IV and B-type natriuretic peptide. from bench to bedside. *Clin. Chem. Lab. Med.* **47**, 248–252 (2009).
264. Sato, Y. *et al.* Role of dipeptidyl peptidase IV (DPP4) in the development of dyslipidemia: DPP4 contributes to the steroid metabolism pathway. *Life Sci.* **88**, 43–49 (2011).
265. Ishibashi, Y., Nishino, Y., Matsui, T., Takeuchi, M. & Yamagishi, S. Glucagon-like peptide-1 suppresses advanced glycation end product-induced monocyte chemoattractant protein-1 expression in mesangial cells by reducing advanced glycation end product receptor level. *Metabolism* **60**, 1271–1277 (2011).
266. Ojima, A. *et al.* Glucagon-like peptide-1 receptor agonist inhibits asymmetric dimethylarginine generation in the kidney of streptozotocin-induced diabetic rats by blocking advanced glycation end product-induced protein arginine methyltranferase-1 expression. *Am. J. Pathol.* **182**, 132–141 (2013).
267. Camara, N. O., Iseki, K., Kramer, H., Liu, Z. H. & Sharma, K. Kidney disease and obesity: epidemiology, mechanisms and treatment. *Nat. Rev. Nephrol.* **13**, 181–190 (2017).
268. Fadini, G. P. *et al.* The oral dipeptidyl peptidase-4 inhibitor sitagliptin increases circulating endothelial progenitor cells in patients with type 2 diabetes: possible role of stromal-derived factor-1alpha. *Diabetes Care* **33**, 1607–1609 (2010).
269. Fujita, H. *et al.* DPP-4 inhibition with alogliptin on top of angiotensin II type 1 receptor blockade ameliorates albuminuria via up-regulation of SDF-1alpha in type 2 diabetic patients with incipient nephropathy. *Endocr. J.* **61**, 159–166 (2014).
270. Jelsing, J. *et al.* Liraglutide: short-lived effect on gastric emptying — long lasting effects on body weight. *Diabetes Obes. Metab.* **14**, 531–538 (2012).
271. Shi, S., Kanasaki, K. & Koya, D. Linagliptin but not sitagliptin inhibited transforming growth factor-beta2-induced endothelial DPP-4 activity and the endothelial-mesenchymal transition. *Biochem. Biophys. Res. Commun.* **471**, 184–190 (2016).

Author contributions

M.H.A.M., L.T., M.M.S., J.A.J. and D.H.vR. researched the data, made substantial contributions to discussions of the content, wrote the article and reviewed and/or edited the manuscript before submission. M.J.B.vB., M.H.H.K. and E.J.H. made substantial contributions to discussions of the content and reviewed and/or edited the manuscript before submission. M.H.A.M. and L.T. contributed equally to this Review.

Competing interests statement

Through M.H.H.K., the VU University Medical Center has received research grants from AstraZeneca, Boehringer Ingelheim, Novo Nordisk and Sanofi. M.H.A.M. and L.T. consulted for Eli Lilly & Co., and D.H.vR. serves on advisory boards of Sanofi Aventis and Merck Sharp & Dohme (all honoraria paid to employer). The other authors declare no competing interests.

Publisher's note

SUPPLEMENTARY INFORMATION

See online article: S1 (table) | S2 (table) | S3 (table) | S4 (figure)

ALL LINKS ARE ACTIVE IN THE ONLINE PDF