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Tonneijck, L.

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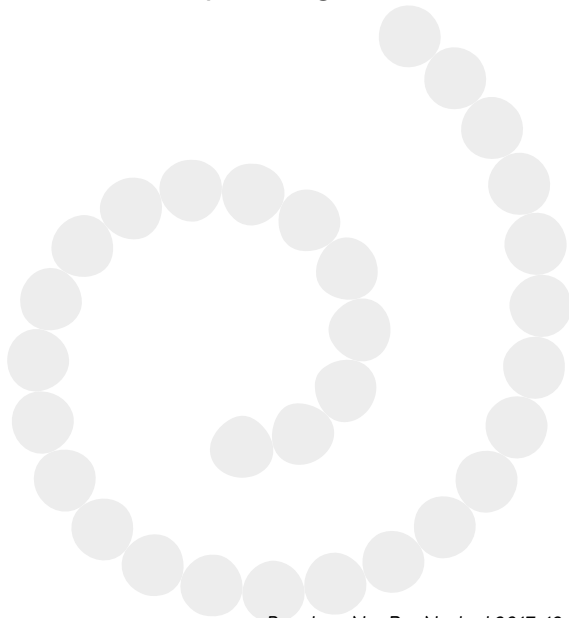
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Summary and general discussion



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In parallel with the global obesity and type 2 diabetes mellitus (T2DM) pandemic,¹ diabetic kidney disease (DKD) has become the most common cause of chronic kidney disease (CKD) and end-stage kidney disease (ESKD).² In addition, DKD increases patients' risk of cardiovascular events and premature death.² DKD is clinically defined as diabetes with albuminuria, impaired renal function (estimated glomerular filtration rate [GFR] <60 mL/min/1.73 m²), or both.³ The prevention and treatment of DKD in T2DM focuses on early detection and control of renal risk factors, including hyperglycaemia, obesity, systemic hypertension, glomerular hyperfiltration, albuminuria, and dyslipidaemia.² With respect to hypertension management, renin-angiotensin system (RAS) inhibitors are widely recommended, as these drugs exhibit blood pressure-independent benefits on renal outcome.⁴ 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.^{5,6} However, in this trial, and also in clinical practice, residual renal risk remains high.^{2,5} Some of this excess risk might be reduced by narrowing the gap between recommended and established risk factor control. In Steno2, a considerable proportion of the participants in the intensive treatment group did not achieve the set targets.⁵ Similarly, from 1999 to 2010, almost half of US adults with diabetes did not meet recommended goals for diabetes care.⁷ Thus, in addition to current therapies, new therapeutic approaches leading to additional risk reduction are needed.

The gut-derived incretin hormone glucagon-like peptide 1 (GLP-1) is secreted upon meal ingestion and lowers glucose levels primarily by modulating pancreatic islet cell function (*i.e.* stimulating glucose-dependent insulin secretion and inhibiting glucagon release), food intake and gastrointestinal motility.⁸ The observation that the insulinotropic actions of GLP-1 are reduced in T2DM led to the development of incretin-based drugs—GLP-1 receptor (GLP-1R) agonists (first in class in 2005) and dipeptidyl peptidase 4 (DPP-4) inhibitors (2007)—for the treatment of hyperglycaemia in these patients.⁸ Considerable interest exists in identifying effects of these drugs beyond glucose-lowering, likely contributing to improved macrovascular outcomes (for some GLP-1R agonists^{9,10}) and microvascular outcomes, including diabetic kidney disease (DKD).¹¹ Indeed, although sporadic cases of acute kidney injury (AKI) in T2DM patients were reported shortly after drug approval,^{12,13} subsequent clinical studies suggested glucose-independent reductions in albuminuria with incretin-based drugs.^{11,14} Several mechanisms by which these drugs affect renal outcome have been proposed. First, GLP-1R agonists and DPP-4 inhibitors favourably affect body weight,¹⁵ blood pressure^{16,17} and dyslipidaemia^{18,19} during long-term treatment. In addition, since 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.¹¹ Indeed, in preclinical studies and small-sized clinical studies, GLP-1R stimulation increased tubular sodium excretion.^{11,20–22} Moreover, GLP-1 and GLP-1R agonists ameliorated glomerular hyperfiltration in rodents,^{23–26} while acute GLP-1 infusion lowered GFR in obese hyperfiltrating males.²⁰ However, the renal effects of clinically used incretin-based drugs in humans were hitherto unknown. Therefore,

the aim of our studies was to determine the effects of GLP-1R agonists and DPP-4 inhibitors on (intra)renal haemodynamics, tubular functions and renal damage markers in healthy males and T2DM patients.

Summary of the main findings

In Chapter 2, we provided an overview of the risk factors of DKD (*i.e.* hyperglycaemia and systemic hypertension, which are the main renal risk factors, in addition to obesity, glomerular hyperfiltration, albuminuria and dyslipidaemia), and discussed favourable and unfavourable glucose-independent actions of widely used antihyperglycaemic drugs on T2DM patients' renal risk profile. We concluded that GLP-1R agonists, and sodium-glucose cotransporter (SGLT)2 inhibitors in particular, seem to show beneficial actions on key risk factors, such as hypertension and obesity, whereas thiazolidinediones and DPP-4 inhibitors might have alternative renoprotective potential beyond glucose-lowering. However, since the available data are based on clinical trials that are mostly placebo-controlled, we emphasised that it is difficult to estimate drug-specific benefits beyond glucose lowering *per se*, as well as to directly compare renal outcomes between glucose-lowering drug classes.

In Chapter 3, we focused on glomerular hyperfiltration as renal risk factor in diabetes. We first defined hyperfiltration at the whole-kidney level and at the single-nephron level. Then we discussed the ultrastructural, vascular and tubular factors that are proposed to underlie this renal haemodynamic abnormality. Next, we summarised available clinical evidence, which suggests that hyperfiltration has a pathogenic and prognostic role in the initiation and progression of DKD. Clearly, dedicated studies with appropriate diagnostic measures and clinically relevant end points are warranted to confirm this postulation. We concluded by discussing several glucose-lowering and other interventions that could attenuate hyperfiltration in diabetes, potentially providing new therapeutic opportunities in alleviating the renal burden in this population.

In Chapter 4, we discussed potential interactions between the incretin- and renin-angiotensin-aldosterone-system (RAAS) pathways, focusing on clinical consequences when pharmacological compounds interfering with these pathways are prescribed simultaneously. First, as GLP-1R agonists have been reported to inhibit circulating levels of angiotensin-II and its post-receptor actions, we speculated that this further increases the protective effects of RAAS inhibitors, or result in augmented risk of adverse events, such as AKI or hyperkalemia. Second, concurrent inhibition of DPP-4 and angiotensin-converting enzyme (ACE) may increase concentrations of substance P and neuropeptide Y (NPY), resulting in augmented sympathetic activity, heart rate and vascular tone. We hypothesise that this response could be involved in the increased incidence in heart failure hospitalisation that was seen in some of the early DPP-4 inhibitor cardiovascular safety trials.

In Chapters 5 to 8, we reported our main findings from the SAFEGUARD (Safety Evaluation of Adverse Reactions in Diabetes) and ELIXIRS (Effect of LIXIsenatide on the Renal System) studies, in which we determined the effects of different GLP-1R agonists or a DPP-4 inhibitor on renal haemodynamics, tubular functions and renal damage markers. In these studies, we

determined glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) by gold-standard inulin and para-aminohippurate clearance techniques, respectively, based on timed urine sampling. We calculated filtration fraction (FF), and estimated intrarenal haemodynamics, *i.e.* glomerular hydrostatic pressure (P_{GLO}) and afferent and efferent arteriole arteriolar resistance (R_{A} and R_{E} , respectively). Tubular functions were assessed by, amongst others, measuring the urinary excretion (UE) of sodium and other electrolytes, as well as urine pH. Renal damage was determined by the urinary albumin-to-creatinine ratio (UACR), the neutrophil gelatinase-associated lipocalin (NGAL)-to-creatinine ratio, and the kidney injury molecule-1 (KIM-1)-to-creatinine ratio.

In Chapter 5, we determined the renal effects of GLP-1R agonist exenatide infusion versus placebo in 10 healthy overweight males. In this open-label, non-randomised study, exenatide increased GFR (+18 mL/min/1.73 m²; +20%), ERPF (+68 mL/min/1.73 m²; +14%) and P_{GLO} (+6%), and decreased R_{A} (-33%). These effects were blunted during concurrent infusion with the nitric oxide (NO)-synthase inhibitor L-N^G-monomethyl arginine (LNMMA). Exenatide also increased absolute and fractional sodium excretion, urine osmolality and urine pH, which were not altered by LNMMA-infusion. These results indicate that immediate exenatide administration induces hyperfiltration under physiological conditions, probably by reducing R_{A} , and at least partially in an NO-dependent manner. Moreover, acute exenatide increases urinary sodium excretion and urine pH, which is likely due to inhibition of the Na⁺/H⁺-exchanger isoform-3 (NHE3) in the proximal tubule.

In Chapter 6, we assessed the renal effects of acute exenatide versus placebo infusion in 52 overweight insulin-naïve T2DM patients with normal renal function. In this double-blind, randomised clinical trial (RCT), exenatide did not change GFR, ERPF, FF, P_{GLO} or R_{E} , but increased R_{A} . Moreover, exenatide increased fractional sodium and potassium excretion, urine osmolality and urine pH, while fractional urea excretion, urinary flow and free water clearance decreased. Osmol clearance, renal damage markers and plasma renin concentrations (PRC) were not altered. Of note, blood pressure was higher in the exenatide-arm. Our results suggest that exenatide does not acutely affect renal haemodynamics in T2DM with normal renal function. Furthermore, the exenatide-induced augmentation in urinary sodium excretion and urinary pH is likely due to a direct GLP-1R-mediated inhibition of NHE3 activity in the proximal tubule, although a role for pressure natriuresis cannot be excluded.

In Chapter 7, we randomised 55 overweight insulin-naïve T2DM patients with normal renal function to receive the DPP-4 inhibitor sitagliptin, the long-acting GLP-1R agonist liraglutide, or matchings placebos in a double-blind RCT. After 12 weeks, neither treatment affected GFR, ERPF, FF, R_{A} or R_{E} . However, only sitagliptin, modestly reduced P_{GLO} (-2.8 mmHg), which was most likely explained by concurrent small reductions in GFR and plasma albumin concentrations used in the formula to calculate this parameter. Moreover, after 12 weeks, neither treatment affected absolute or fractional excretion of sodium, urea and potassium excretion, or renal damage makers or PRC. After 2 weeks, sitagliptin increased fractional excretion of sodium and urea. Thus, prolonged treatment with sitagliptin or liraglutide in overweight T2DM patients with normal renal function does not affect renal haemodynamics. Also, no sustained changes

in tubular functions or alterations in renal damage markers were observed. The validity and clinical relevance of the sitagliptin-induced P_{GLO} reduction remains speculative.

In Chapter 8, we assessed the renal effects of 8-week treatment with the short-acting GLP-1R agonist lixisenatide once-daily versus titrated insulin glulisine once-daily following a standardised breakfast. In this open-label RCT in 35 overweight insulin glargine-treated T2DM patients without overt nephropathy, lixisenatide did not change GFR, ERPF, FF or intrarenal haemodynamics. Fractional sodium excretion and urine pH increased with lixisenatide, which seemed—at least in part—to depend on a post-breakfast vasopressor response in regression analyses. We did not observe changes in renal damage markers, PRC, angiotensin-II or aldosterone. These results indicate that prolonged lixisenatide treatment does not affect post-breakfast (intra-)renal haemodynamics compared with insulin glulisine in T2DM patients without overt nephropathy. Prolonged lixisenatide treatment has a sustained natriuretic effect, which contrasts with long-acting GLP-1R agonists, and it increases postprandial blood pressure.

In Chapter 9, we performed a secondary analysis of the latter trial to further detail the magnitude and mechanisms by which lixisenatide affects electrolyte homeostasis in T2DM. After a standardised breakfast, lixisenatide compared to insulin glulisine increased absolute and fractional excretions of sodium, chloride, and potassium and increased urinary pH. In contrast, lixisenatide reduced absolute and fractional excretions of magnesium, calcium, and phosphate. At Week-8, patients treated with lixisenatide had significantly more phosphorylated NHE3 in urinary extracellular vesicles than those on insulin glulisine treatment, suggesting decreased NHE3 activity. Moreover, the increase in post-breakfast blood pressure with lixisenatide partly explained the changes in the urinary excretion of sodium, potassium, magnesium and phosphate, and urinary pH. Thus, lixisenatide affects postprandial urinary excretion of several electrolytes and urinary pH compared to insulin glulisine after 8 weeks of treatment. This is most likely explained by blood pressure-mediated or direct inhibitory effects on NHE3 in the proximal tubule.

In Chapter 10, we performed another secondary analysis of the same trial to detail the post-breakfast blood pressure response of lixisenatide, and to explore underlying mechanisms that could drive blood pressure changes. Here, we showed that lixisenatide compared to insulin glulisine does not affect fasting, but increases post-breakfast systolic blood pressure (overall by 5.2 mmHg and maximally by 10.2 mmHg), diastolic blood pressure (overall by 5.4 mmHg and maximally by 7.2 mmHg), systemic vascular resistance and arterial stiffness. We did not observe between-group differences in overall post-breakfast cardiac output or cardiac sympathovagal-balance, and concentrations of angiotensin-II, aldosterone of catecholamines. Interestingly, the slowing of gastric emptying rate with lixisenatide statistically explained changes in post-breakfast blood pressure. This suggests that lixisenatide may—amongst others— increase post-breakfast blood pressure by reducing the passage rate of nutrients and water, and by activating the gastrovascular reflex through gastric distension.

In Chapter 11, we reported *post-hoc* analyses of four clinical trials to determine the effects of GLP-1R agonists on uric acid (UA) levels (an emerging cardiovascular and renal risk factor) and its renal clearance. We showed that acute exenatide infusion increases urinary UA excretion in

healthy overweight males and overweight T2DM patients. Since these changes were statistically related to increases in urinary sodium excretion and urine pH, we suggested that the UA response is mediated by GLP-1R agonist-induced inhibition of NHE3 activity and/or consequent urine alkalinisation. However, prolonged treatment with the long-acting GLP-1R agonist liraglutide or the short-acting GLP-1R agonist lixisenatide did not affect urinary UA excretion or UA levels in overweight T2DM patients, indicating that these immediate actions are subject to receptor tachyphylaxis and/or induction of (tubular) compensatory mechanisms.

In Chapter 12, we responded to a prespecified secondary analysis of the landmark LEADER trial, which provided details on a previously reported 22% reduction in the composite renal outcome with long-term use of liraglutide compared to placebo in 9340 T2DM patients. As the contribution of liraglutide-induced improvements in HbA_{1c}, bodyweight and blood pressure were not clear in this study, leaving uncertainty regarding a potential drug-specific benefit, we urged the authors to perform additional analyses. In response, they adjusted the composite renal outcome for the change in HbA_{1c}, body weight, and systolic blood pressure, separately and together, which yielded similar results. These results suggest that liraglutide confers renoprotection independent from reductions in glucose, bodyweight and/or blood pressure, hinting towards other renoprotective pathways of the drug, such as direct inhibition of systemic or tissue inflammation.

In the following section, we will integrate our findings with the present literature to provide a comprehensive and in-depth up-to-date overview regarding the renal effects of incretin-based drugs. First, we will discuss *direct* pathways by which incretin-based may affect the kidney. We will start with a discussion of the tubular effects through which (some of) the renal haemodynamic effects may be mediated. Then, we will discuss other off-target effects through which incretin-based drugs may *indirectly* reduce DKD-burden. Finally, we will bridge these mechanistic insights to clinical practice by reviewing the effects of GLP-1R agonists and DPP-4 inhibitors on renal outcomes in T2DM patients, based largely on studies that became available very recently and/or concurrently with the more mechanistic studies.

GENERAL DISCUSSION

Direct renal effects of incretin-based drugs

Tubular effects

With regard to GLP-1R agonists, acute intravenous infusion of exenatide in healthy males (Chapter 5)²⁷ and patients with T2DM (Chapter 6),²⁸ and a single subcutaneous injection of liraglutide in patients with T2DM,²⁹ 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,³⁰ but the results of our placebo-controlled liraglutide study in insulin-naïve patients with T2DM suggest that this response

disappears before week 12 (Chapter 7).³¹ This loss of effect is compatible with the GLP-1R desensitisation that has been observed for other regulatory systems after prolonged treatment with a long-acting GLP-1R agonist. Alternatively, it might indicate compensatory activation of renal sodium transporters or reduced dietary sodium intake. Interestingly, our data 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 (Chapter 8) (Figure 1).³² DPP-4 inhibitors also promote natriuresis, as demonstrated in animals^{26,33,34} and in patients with T2DM after 2–4 weeks of sitagliptin treatment (this effect disappeared before week 12) (Chapter 7).^{31,35}

GLP-1R-mediated natriuresis and diuresis seem to involve inhibition of NHE3 (Figure 1), which is located at the brush border of the renal proximal tubule bound to a complex that also contains DPP-4.^{36,37} As measured in renal tissue of rodents, 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,^{33,38,39} which reduces its activity. Conversely, GLP-1R blockade with exendin 9 reduces renal cortical phosphorylation at Ser552 of NHE3.³⁹ In animals and humans, acute GLP-1R agonist infusion increases renal lithium clearance (a marker of proximal tubular sodium reabsorption)^{21,29,33,40} and urine pH (Chapter 5 and Chapter 6),^{20,27,28} supporting the notion that NHE3 is indeed involved. As acute GLP-1R agonist administration increases fasting mean arterial pressure by ~5 mmHg in most reported human studies (through incompletely understood mechanisms; Chapter 5 and Chapter 6),^{27–29,41} pressure natriuresis (characterized by a rapid coordinated decrease in both NHE3 surface distribution and Na⁺/K⁺ ATPase activity⁴²) might be at least partly be involved in the natriuretic effect. Also, in T2DM patients, a post-breakfast vasopressor response partly explained the natriuretic effect of 8 week lixisenatide treatment in statistical analyses (Chapter 8 and Chapter 9).^{32,43} Notably, in a subgroup of patients of the latter trial, increased NHE3 phosphorylation at Ser552 was observed in the lixisenatide- compared to the insulin glulisine treated patients (Chapter 9).⁴³ DPP-4 inhibitors have also been suggested to inhibit NHE3 activity, albeit through a tyrosine kinase signalling pathway rather than via PKA activation.³⁷ Furthermore, DPP-4 inhibitors might redistribute NHE3 (along with a small fraction of DPP-4),³⁶ and stimulate NHE3-independent sodium excretion (see below).^{11,44}

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, glucagon, ATP and glucose itself, regulate NHE3 and SGLTs in the kidney, suggesting indirect natriuretic actions of GLP-1 (Figure 1).⁴⁵ Moreover, interaction of GLP-1R agonists with (other) 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.¹¹

First, GLP-1R agonists might reduce circulating levels and post-receptor actions of components of the RAAS (Chapter 4).^{46,47} GLP-1 infusion in healthy young males,²¹ and a single-dose of liraglutide in patients with T2DM,²⁹ decreased plasma levels of angiotensin-II by ~20%. Similarly, GLP-1 infusion reduced plasma renin activity in healthy males⁴⁸ and obese

insulin-resistant males.²⁰ However, we and others were unable to confirm effects of acute or prolonged GLP-1R agonist treatment (Chapter 6, Chapter 7 and Chapter 8)^{28,30-32,48-50} or DPP-4 inhibitor therapy (Chapter 7)^{31,35} on circulating RAAS components (Supplemental Table 1). Second, an indirect role for neural pathways might be involved, as intra-cerebroventricular GLP-1 administration also rapidly stimulates UE of water and sodium.²² Third, a stimulating effect on natriuretic peptides should be considered. Although a gut–cardiac GLP-1R–atrial natriuretic peptide (ANP) axis was identified in nondiabetic rodents,⁵⁵ subsequent studies of the effects of GLP-1 infusion in healthy males^{48,56} and of short-term liraglutide therapy in patients with T2DM,^{29,30} 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,

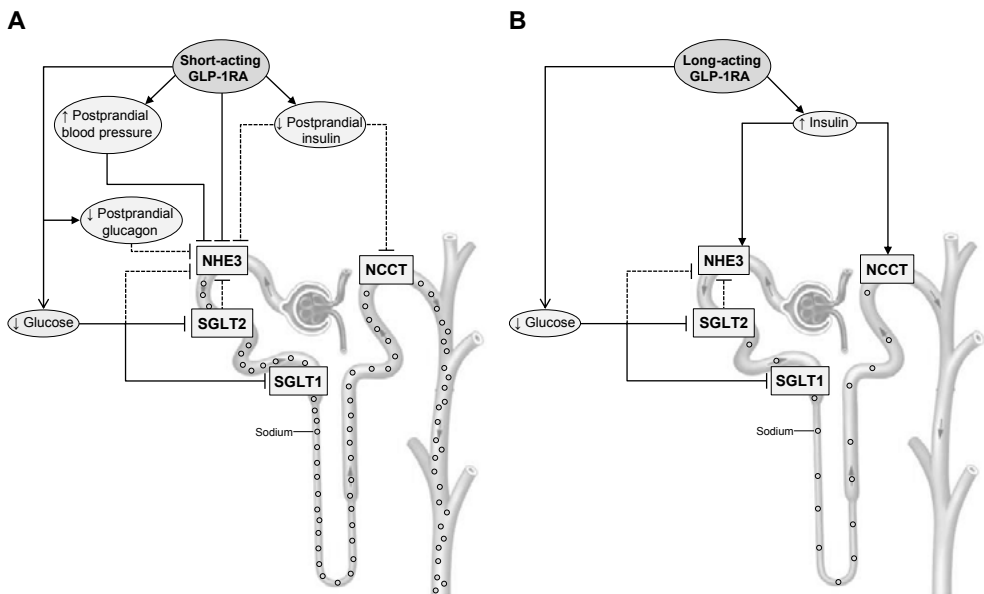


Figure 1. Putative natriuretic mechanisms of GLP-1RAs. (A) Immediate glucagon-like peptide-1 receptor agonist (GLP-1RA) administration or prolonged treatment with a short-acting GLP-1RA may induce natriuresis by inhibiting Na⁺/H⁺-exchanger isoform-3 (NHE3) activity, either directly or indirectly through a (postprandial) vasopressor response. NHE3 could also be inhibited through reduced levels of postprandial 1) glucagon, leading to inhibition of NHE3 in thick ascending limb^{51,52} and potentially in the proximal tubule,^{52,53} 2) insulin,⁵⁴ leading to inhibition of NHE3 and Na⁺/Cl⁻ cotransporters (NCCT) in the distal convoluted tubule, 3) and/or glucose, leading to downregulation and inhibition of NHE3 and sodium-glucose cotransporters (SGLT) 1 and 2.¹¹ SGLT2 and NHE3 may be functionally linked such that SGLT2 inhibition may also inhibit NHE3.⁵⁴ However, statistical analyses do not indicate that short-acting GLP-1RA induced changes in glucagon, insuline and/or glucose (dashed lines) affect NHE3 (Chapter 9).⁴³ Potential neuronal pathways, or less likely effects (during clinical use) through atrial natriuretic peptide, angiotensin-II or aldosterone are not depicted. (B) Prolonged treatment with long-acting GLP-1RAs does not induce natriuresis, potentially due to receptor tachyphylaxis, induction of (tubular) compensatory mechanisms, limited renal filtration of metabolites and/or reduced dietary sodium intake with these agents.

administration, suggesting that the natriuretic effects of DPP-4 inhibitors are in part GLP-1-independent.^{26,44} Elevated levels of intact stromal cell-derived factor 1 α (SDF1 α) might mediate this DPP-4-inhibitor-induced natriuretic response, likely via Na⁺/Cl⁻ cotransporters (NCCT) or the epithelial sodium channel (ENaC) in the distal convoluted tubule.²⁶

A placebo-controlled RCT in 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 SDF1 α levels and a decrease in truncated SDF1 α levels.³⁵ However, other DPP-4 substrates have also been associated with RAAS interaction and renal sodium excretion, including NPY, peptide YY (PYY), substance P (Chapter 4)⁴⁶ and brain natriuretic peptide.¹¹ These substrates might contribute to the GLP-1-independent tubular effects of DPP-4 inhibitors.¹¹

In addition to natriuresis and diuresis, GLP-1R agonists have been reported to increase free water clearance in hyperhydrated rats^{57,58} and humans,⁵⁹ as well as absolute and fractional excretion of potassium (Chapter 6),^{28,33,34,40} chloride,^{20,34,44,60} calcium^{20,21} and UA (Chapter 11)⁶¹ in the acute setting (Supplemental Table 1). Prolonged liraglutide treatment did however not affect UE of potassium, urea (Chapter 7)³¹ or UA (Chapter 11).⁶¹ In contrast, 8-week treatment with lixisenatide following the breakfast, but not in the fasting state, increased UE of chloride and potassium, reduced UE of magnesium, calcium and phosphate, and did not affect UE of urea (Chapter 9)⁴³ or UA (Chapter 11).⁶¹ The lixisenatide-induced changes in UE of electrolytes could, in theory, be explained by NHE3-inhibition, through coupling with chloride transporters in the proximal tubule and secondary sodium-dependent effects on downstream tubular transporters of potassium, magnesium, calcium and phosphate (Chapter 9).⁴³

Renal 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 GFR³⁹ (increased proximal tubular reabsorption should inhibit tubuloglomerular feedback signals, leading to reduced afferent arteriolar resistance and increased GFR [Chapter 3]⁶²).

The physiological role of GLP-1-induced glomerular hyperfiltration might be to enhance the filtered electrolyte load after meal ingestion.⁴⁴ Single-nephron GFR was acutely raised by up to 50% in rats without diabetes after GLP-1 infusion^{34,40,63} or administration of exenatide⁶⁴ or liraglutide.³⁴ Similar, albeit smaller, GFR increases in nondiabetic rodents have been observed with DPP-4 inhibitors.^{33,34,39} Acute GLP-1 infusion did not affect GFR or ERPF in healthy men,^{21,48} but exenatide infusion increased GFR, ERPF and P_{GLO} in overweight but otherwise healthy males (Chapter 5).²⁷ A direct vasodilatory effect on the afferent renal arteriole (through GLP-1R-dependent actions on vascular smooth muscle cells) may be involved (Chapter 5).^{27,63} In rodents, GLP-1R blockade with exendin 9 attenuated GLP-1-induced increases in renal blood flow and reductions in autoregulatory responses to increased R_A of isolated murine kidneys,⁶³ while GLP-1 infusion increased renal blood flow and induced renal arterial vasodilation.⁴⁵ In addition, in healthy males, exenatide infusion acutely reduced R_A, which seems to be, at least in

part, dependent on NO availability (Chapter 5).²⁷ These findings imply that GLP-1R agonists are proximal diuretics and renal vasodilators that under healthy conditions only mildly influence tubuloglomerular feedback.⁶⁴

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,⁴⁴ and clinical studies suggest that incretin-based drugs reduce GFR and may ameliorate glomerular hyperfiltration.^{20,50,65,66} 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 (Chapter 3)⁶² that are allegedly inhibited by GLP-1R agonists (Figure 2). In the setting of insulin resistance or diabetes, the magnitude of the direct vasodilator effect of GLP-1R might be reduced owing to impaired NO-dependent vasodilation,

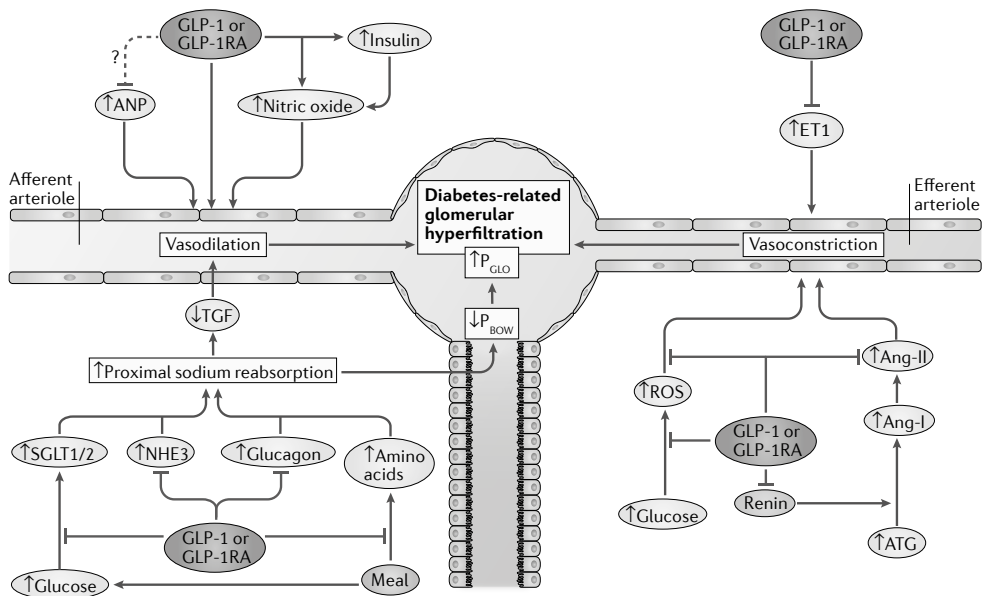


Figure 2. Effects of glucagon-like peptide 1 (GLP-1) and GLP-1 receptor agonists (GLP-1RAs) on renal haemodynamics in diabetes mellitus. Several vascular and tubular factors are implicated in fasting and postprandial glomerular hyperfiltration in the setting of diabetes. These factors result in a net 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 (which may be modified by an increase in blood pressure in some settings; not depicted) and inhibition of pathways of glomerular hyperfiltration. Theoretically, this effect is dependent on baseline phenotypic characteristics and comedication. AngI, angiotensin I; AngII, angiotensin II; ANP, atrial natriuretic peptide; ATG, angiotensinogen; ET1, endothelin 1; NHE3, Na^+/H^+ -exchanger isoform-3; ROS, reactive oxygen species; SGLT, sodium-glucose cotransporter; TGF, tubuloglomerular feedback.

whereas the inhibitory role of GLP-1 on factors associated with glomerular hyperfiltration could be substantial. The response may additionally be modified by augmentation of blood pressure in the acute setting or upon prolonged treatment with short-acting GLP-1R agonists (Chapter 6 and Chapter 8).^{28,32} 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 [Chapter 3]⁶²) might be an essential milieu for incretin-based drugs to confer renoprotective alterations in renal haemodynamics.⁶⁷ 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.⁶⁸

Indeed, in early studies in diabetic rodents, 4–8 weeks of administration of exendin 4,^{24,25} liraglutide²³ or linagliptin²⁶ 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 obese insulin-resistant males, which was in contrast to neutral effects observed in healthy subjects (Supplemental Table 1).²⁰ In an uncontrolled study in patients with T2DM, liraglutide was associated with an acute reduction in eGFR (paralleled by a reduction in albuminuria) and subsequent stabilisation up to 49 days of therapy, which was reversible after 3 week washout of the drug, suggesting a renal haemodynamic effect.⁶⁶ Reinitiation of liraglutide therapy in a 1 year extension of the trial showed a similar eGFR pattern.⁶⁵ 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 (Chapter 7),³¹ reminiscent of the effects of ACE inhibitors and SGLT2 inhibitors (Chapter 3),⁶² the eGFR trajectory did not differ from placebo. In RCTs of 12–30 weeks duration, GLP-1R agonist or DPP4 inhibitor treatment did not influence initial changes in or the slope of eGFR in patients with T2DM with^{69–72} or without^{71,73–75} renal impairment. Moreover, acute administration of GLP-1,⁷⁶ exenatide (Chapter 6)²⁸ or liraglutide,²⁹ or 12 weeks of liraglutide or sitagliptin treatment (Chapter 7)³¹ did not affect fasting renal haemodynamics or P_{GLO} in patients with T2DM and presumed late-phase hyperfiltration (that is, filtration fraction of ~25% in the setting of $\text{GFR} > 60 \text{ ml/min/1.73 m}^2$).⁶² 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 phase hyperfiltration ($\text{GFR} > 135 \text{ ml/min/1.73 m}^2$).⁵⁰ Furthermore, 4 weeks of linagliptin therapy versus placebo⁷⁷ or 8 weeks of lixisenatide therapy versus insulin glulisine (Chapter 8)³² in patients with T2DM did not affect fasting or postprandial renal haemodynamics, respectively. Notably, in studies in T2DM patients with normal kidney function involving acute exenatide-infusion, (Chapter 6)²⁸ or prolonged treatment with liraglutide, sitagliptin (Chapter 7),³¹ or lixisenatide (Chapter 8),³² correlative analyses did not suggest that these drugs reduce GFR or P_{GLO} in patients with higher baseline GFR, FF or P_{GLO} (unpublished data). Finally, one-year treatment with exenatide twice-daily did not affect 24 h creatinine clearance or estimated GFR-trajectory compared to titrated insulin glargine in T2DM without over nephropathy.⁷⁸ In conclusion, GLP-1R agonists seem to induce glomerular hyperfiltration under physiological conditions by reducing R_A , but maintain or

might slightly improve renal haemodynamic function in patients with T2DM, likely depending on the individual baseline phenotypic characteristics, and potentially affected by concurrent blood pressure modification (Figure 2).

Indirect renoprotective effects of incretin-based drugs

Body weight and composition

Prolonged treatment with GLP-1R agonists is associated with reductions in body weight (Chapter 7 and Chapter 8)^{31,32,79} and waist circumference, albeit with much variation in individual responses and within class differences.⁷⁹ By contrast, DPP-4 inhibitors do not affect body weight (Chapter 7).^{15,31} In a 2015 meta-analysis⁸⁰ 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).¹⁵ Of note, liraglutide-induced weight loss is dose-dependent up to 3.0 mg once daily. A study in 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.⁸¹ 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.⁸² 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.^{83,84} This effect seems to be the result of a GLP-1R-mediated reduction in appetite and food intake, rather than of increased energy expenditure.⁸⁵

Several DPP-4 substrates are pharmacological regulators of food intake (for example PYY, NPY and GLP-2), but unlike GLP-1R agonists, DPP-4 inhibitors do not suppress 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 1).

Blood pressure

Acute administration of GLP-1R agonists increases heart rate and leads to a transient increase in fasting blood pressure in normotensive and hypertensive individuals (Chapter 6).^{28,29,41,48,86,87} Sustained treatment with GLP-1R agonists in patients with T2DM is, however, associated with a persistent increase in heart rate, while both incretin-based drug classes lead to a clinically relevant drop in blood pressure (Chapter 7),^{16,17,31} which is not strictly dependent on weight loss.^{16,17} 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.¹⁶ The blood-pressure-lowering effect of GLP-1R agonists does not seem to be dose-dependent.⁸⁸

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.¹⁷ Mechanisms linking sustained GLP-1R signalling to blood pressure reductions are also incompletely understood, but might include contributions from natriuresis and/or diuresis, improved vasorelaxation and/or endothelial function and neurohormonal pathways (Table 1).

Interestingly, while prolonged lixisenatide decreases blood pressure in the ELIXA trial,⁸⁹ the drug induces a vasopressor response following a standardised breakfast (see above; Chapter 10).⁹⁰ This effect could —at least in-part— be explained by a sustained inhibitory effect on gastric emptying rate (which wanes upon prolonged treatment with long-acting GLP-1R agonists due to receptor tachyphylaxis), leading to a reduced passage rate of nutrients and water, and activation of the gastrovascular reflex through gastric distension (Chapter 10).⁹⁰ Future studies should establish whether this response with lixisenatide, and potentially also with other short-acting GLP-1R agonists, influences cardiovascular risk in individual patients, or could aid in the prevention of postprandial hypotension. Of note, an ongoing proof-of-concept study in 30 T2DM patients is evaluating the potential of lixisenatide in the management of postprandial BP-falls.⁹¹

Dyslipidaemia

Incretin-based drugs modestly contribute to improvement of fasting and particularly postprandial lipid profiles in patients with T2DM. A 2015 meta-analysis of 25 trials with a duration ranging from 8 to 84 weeks showed that GLP-1R agonist therapy can result in small reductions in the levels of low-density lipoprotein (LDL) cholesterol, total cholesterol and triglycerides, but does not improve HDL cholesterol levels in comparison to placebo and active comparators.¹⁹ For DPP-4 inhibitors, a meta-analysis of 53 RCTs suggested a small benefit on total cholesterol levels of ~0.18 mmol/l.¹⁸ Of note, in our RCTs in T2DM patients, we did not observe improvements in fasting lipids after 12 week treatment with liraglutide or sitagliptin compared to placebo (Chapter 7)³¹, or 8 week lixisenatide compared to insulin glulisine treatment (Chapter 8).³² Mechanisms by which GLP-1R agonists may improve dyslipidaemia are only partially understood, but could relate to reduced intestinal chylomicron production and secretion.¹¹⁸ Incretin-based drugs reduced liver fat and decreased hepatic lipoprotein synthesis and secretion in some but not all studies.^{118,119} 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 1). As such, incretin-based drugs 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,¹⁰⁰ 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 (and, in general, cardiovascular) function and morphology. Although not specifically addressed in the present

Table 1. Glucose-independent effects of incretin-based drugs 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	<ul style="list-style-type: none"> ↓ Appetite (direct effect CNS or via vagal afferents, ↓GEE* and ↑nausea) ↑ Energy expenditure⁸⁵? ↑ Natriuresis and/or diuresis? ↓ Body weight ↑ Endothelial independent vasodilation^{94,95}? ↑ Natriuresis⁹⁶ ↓ Intestinal sodium reabsorption⁹⁷? ↓ Sodium intake (direct effect CNS)? ↓ RAAS activity^{46,47}? ↑ ANP⁵⁵? 	<ul style="list-style-type: none"> Effect possibly counteracted by ↑PYY(1-36) and ↓PYY(3-36)^{92,93*} ↑ Natriuresis (↑SDF-1α²⁶, ↓DPP-4/NHE3 complex⁹⁸?, ↑BNP⁹⁹) ↑ Vasodilation (↑BNP⁹⁹, ↑bradykinin) Effects possibly counteracted by ↑substance-P (↑SNS activity) and ↑NPY (potentiates SNS activity) during concomitant ACE-inhibition⁴⁶
Dyslipidaemia	Decrease	Neutral effect	<ul style="list-style-type: none"> ↓ Body weight ↓ Intestinal lipid uptake (partly by ↓GEE*) ↓ Hepatic lipoprotein synthesis and secretion ↑ Insulin sensitivity (partly by ↓bodyweight) ↑ Insulin and ↓glucagon ↑ Triglyceride uptake in white adipose tissue ↑ Brown adipose tissue activation¹⁰⁰ 	<ul style="list-style-type: none"> Effects possibly counteracted by factors related to steroid metabolism¹⁰¹
Inflammation and fibrosis	Decrease	Decrease	<ul style="list-style-type: none"> ↓ Renal ROS production (cAMP and PKA)^{33,102} ↓ AGE-RAGE-mediated renal ROS production (cAMP)¹⁰³⁻¹⁰⁵ ↓ Angiotensin-II-induced renal ROS production (PKC)^{106,107} ↑ Adiponectin (reduces podocyte inflammation; PKA in adipocytes)¹⁰⁸ 	<ul style="list-style-type: none"> ↑ SDF-1α^{26,109,110} ↓ Profibrotic endothelial-to-mesenchymal transition^{111,112*}

Table 1. (continued)

Renal risk factor	GLP-1RA or DPP-4 inhibitor	Putative GLP-1-mediated mechanisms	Putative GLP-1-independent mechanisms of DPP-4 inhibitors
Glomerular hyperfiltration	Decrease or neutral effect	<ul style="list-style-type: none"> ↑ Tubuloglomerular feedback (by ↓NHE3 activity) ↓ Postprandial glucagon (particularly short-acting GLP-1RA)^{62,113,114?} ↓ Body weight^{62?} ↓ GEE* (postprandial hyperfiltration)^{62?} ↓ RAAS activity^{46,47?} 	<ul style="list-style-type: none"> ↑ SDF-1α^{26?}

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, SDF1 α , stromal cell-derived factor 1 α . *GEE reduction is subject to tachyphylaxis after prolonged treatment with long-acting GLP-1RA; however, loss of body weight continues.^{85,115} †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^{81,82}; 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 glycolyx. ††An ongoing trial is investigating this hypothesis in detail.¹¹⁶ †††This effect could be drug-specific as lixisenatide, but not sitagliptin, reduces endothelial-to-mesenchymal transition.¹¹⁷ ††††Lixisenatide, and potentially also other short-acting GLP-1RAs, increases postprandial blood pressure.^{32,90}

thesis, 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.¹¹⁸ Preclinical studies report that incretin-based drugs inhibit inflammatory signalling pathways of DKD in a glucose-independent manner, commonly paralleled by reductions in albuminuria and improved histological features of diabetic nephropathy (Supplemental Table 2). Notably, in some studies that claimed glycaemic equipoise in intervention and placebo groups,^{23,120-125} 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/TaAkita diabetic mice²³ and in rats with streptozotocin-induced diabetes¹⁰² 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.¹⁰² GLP-1R-induced cAMP activation might also result in reduced expression of the receptor for advanced glycation end products, resulting in anti-oxidative effects.¹⁰⁵

In addition to GLP-1R-mediated effects,¹²³ the glucose-independent anti-inflammatory and antifibrotic actions of DPP-4 inhibitors could be mediated by other DPP-4 substrates¹¹ or by direct mechanisms, as DPP-4 has roles in T cell development, T cell activation and immune regulation.¹²⁶ For example, the DPP-4 substrate SDF1 α has a central role in cell and tissue repair under conditions of vascular occlusion and ischaemic renal injury.²⁶ Thus, linagliptin-induced expression of SDF1 α 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 mice²⁶ and rats.¹²⁷ Linagliptin also reduced renal fibrosis by directly inhibiting transforming growth factor- β (TGF β)-induced endothelial-to-mesenchymal transition in T1DM mice models.^{111,112} The mechanism could potentially involve suppression of hyperglycaemia-induced interactions of DPP-4 and cation-independent mannose-6-phosphate receptor¹²⁸ and/or DPP-4 and integrin β 1.¹²⁹

Glucose-independent anti-inflammatory and antioxidant actions of incretin-based drugs 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 glargine⁸³ or glibenclamide¹³⁰ reduced the levels of high-sensitivity C-reactive protein by 25–60% in patients with T2DM, independent of glucose, body weight and body fat.⁸³ 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.¹³¹ Finally, in microalbuminuric patients with T2DM, 16 weeks of treatment with exenatide twice daily reduced UE of TGF β 1 and type IV collagen compared to glimepiride, despite greater glycaemic improvements with glimepiride.¹³²

Renal protection: from risk factors to outcome

“Smaller”-sized clinical studies and pooled analyses

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^{65,66,133,134} and halted eGFR decline over a period of 12 months.^{133,134} 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,⁸⁸ 56 weeks of treatment with liraglutide 3.0 mg, liraglutide 1.8 mg or placebo resulted in reductions in UACR of 18.4%, 11.8% and 2.3%, respectively, suggesting dose-dependent effects of liraglutide on albuminuria. In the LIRA-RENAL trial,⁷⁰ patients with T2DM and moderate renal impairment (eGFR 30–59 ml/min/1.73 m²) were randomly assigned to liraglutide 1.8 mg or placebo for 26 weeks. At study end, the intervention group showed a nonsignificant improvement in UACR relative to baseline (0.83, 95%-CI 0.62–1.10; p=0.19) but 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 daily⁷⁴ and dulaglutide⁷⁵ 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,⁵⁰ 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.⁵⁰

Headtohead trials of GLP-1R agonists with active comparators can directly determine 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 increased UACR by 104 mg/g versus a decrease of 47 mg/g, although the between-group difference was not significant.¹³⁵ 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).⁷⁵ 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 1year treatment period (0.26% versus 1.25%; p=0.012).⁷⁵ The available data from the 26week AWARD7 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.¹³⁶ Moreover, in patients with T2DM, 26 weeks of exenatide therapy reduced HbA_{1c} levels to a similar extent as gliclazide, but resulted

in a greater decrease in 24 h urinary albumin excretion (38.0% versus 5.8%).¹³² 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 (Chapter 8).³² Also, among metformin-treated T2DM patients without overt nephropathy, one-year treatment with exenatide twice-daily did not affect 24 h albuminuria compared to titrated insulin glargine.⁷⁸ Thus, these studies suggest that, particularly in patients with DKD, GLP-1R agonists may modestly improve albuminuria, which seems to be partially independent of glycaemic control.

DPP-4 inhibitors

Increased serum and renal DPP-4 activity is associated with albuminuria and DKD.¹³⁷⁻¹⁴² 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.¹⁴³⁻¹⁴⁶ 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%; $p=0.02$).⁷³ This outcome was driven by an 18% reduction in moderate and 14% reduction in severe elevations of albuminuria with no significant effects on eGFR. 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.¹⁴⁷ The MARLINAT2DTM trial in 360 patients with T2DM on stable RAAS inhibition,¹⁴⁸ 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.¹⁴⁸ The CARMELINA trial,¹⁴⁹ 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.¹⁵⁰ Interestingly, the sitagliptin-induced UACR reduction correlated with changes in diastolic blood pressure, but not in HbA_{1c} levels.¹⁵⁰ Finally, a RCT in 170 patients with T2DM showed that sitagliptin and exenatide once weekly both reduced UACR compared to pioglitazone after 26 weeks.¹⁵¹

Thus, these studies indicate that DPP-4 inhibitors modestly improve albuminuria, plausibly beyond glycaemic control. A more dedicated study in T2DM patients is ongoing to determine effects on hard renal end points.

Renal outcomes in large cardiovascular studies

Licensing of glucose-lowering 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.¹⁵² Seven large randomised cardiovascular outcome studies involving incretin-based drugs have now reported their primary results. ELIXA,⁸⁹ LEADER,¹⁵³ SUSTAIN-6¹⁵⁴ and EXSCEL¹⁵⁵ examined the cardiovascular safety of the GLP-1R agonists lixisenatide (N=6,068), liraglutide (N=9,340), semaglutide (N=3,297) and exenatide once-weekly (N=14,752) for 25 months, ~3.8 years, 2.1 years and 3.2 years, respectively. In addition, SAVOR-TIMI 53,¹⁵⁶ EXAMINE¹⁵⁷ and TECOS¹⁵⁸ studied 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. 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 three-point major adverse cardiovascular events (MACE; cardiovascular death, nonfatal MI or nonfatal stroke) or four-point MACE (also including hospitalisation for unstable angina). As reviewed in depth elsewhere,¹⁴ all three DPP-4 inhibitor outcome studies and the ELIXA trial achieved the non-inferiority margin specified by the FDA in their primary composite outcome, suggesting that the drugs are cardiovascular neutral. For liraglutide and semaglutide, a remarkable reduction in three-points MACE was demonstrated, of 13% ($p=0.01$)¹⁵³ and 26% ($p=0.02$),¹⁵⁴ respectively, while an apparent near miss with respect to statistical significance was seen with exenatide once-weekly (9%, $p=0.06$).¹⁵⁵ All studies incorporated secondary and exploratory renal end points, which will be discussed below (Table 2).

GLP-1R agonists

In the ELIXA trial,⁸⁹ 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.⁸⁹

Both LEADER and SUSTAIN6 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/1.73 m²; 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,^{153,159} whereas 104 weeks of semaglutide treatment reduced this composite outcome by 36%.¹⁵⁴ Notably, the renal benefit in LEADER was predominantly driven by a 26% reduction in macroalbuminuria, whereas in SUSTAIN6, an even larger 46% reduction in macroalbuminuria seems exclusively responsible for the favourable renal outcome of semaglutide.¹⁵⁴ In LEADER, liraglutide also 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/1.73 m² at baseline.¹⁵⁹

The EXSCEL trial¹⁵⁵ only reported the prevalence of albuminuria and ESRD during the overall study period, which was lower in the exenatide once-weekly versus the placebo

group (microalbuminuria, 7.2% versus 7.5%; macroalbuminuria, 2.2% versus 2.8%; ESRD 0.7% versus 0.9%).¹⁵⁵

Given the glucose-dependency of the albuminuria effect in ELIXA, and the fact that differences in HbA_{1c} levels in LEADER, SUSTAIN6 and EXSCEL were even more substantial (time-averaged mean compared to placebo of ~0.6%, ~1.1%, and ~0.5% respectively), the potential impact of glycaemic control should be recognized.¹⁶⁰ Also, modest improvements in body weight (~2–4 kg) and systolic blood pressure (~1–3 mmHg), may have played part in the renoprotective effects of the drugs. Interestingly, recent additional analyses in LEADER, in which the risk of the composite renal outcome was adjusted in a time-dependent manner for the change from baseline in HbA_{1c}, body weight, and systolic blood pressure, separately and together, resulted in similar results (Chapter 12).¹⁶¹ Notably, the differences in serum lipid levels and the estimated GFR between the groups were not accounted for since these were small throughout the trial. Although such analyses in a trial with many potential time-varying confounders is not easily performed, it suggest that liraglutide confers renoprotection independent from reductions in glucose, bodyweight and/or blood pressure, hinting towards other renoprotective pathways of this drug (and potentially also other GLP-1R agonists), such as direct inhibition of systemic or tissue inflammation (Chapter 12).¹⁶¹

DPP-4 inhibitors

In SAVOR-TIMI 53, more patients with T2DM in the saxagliptin group than in the placebo group shifted to a lower UACR category at the end of the trial, irrespective of baseline UACR category (p=0.021 for normoalbuminuria, p<0.001 for microalbuminuria and p=0.049 for macroalbuminuria).^{156,162} At 2 years, 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.^{156,162} 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 m², -19 mg/g; eGFR 30–50 ml/min/1.73 m², -105 mg/g; eGFR<30 ml/min/1.73 m², -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.¹⁶² 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 2).

EXAMINE only reported change in eGFR from baseline, which was similar in the alogliptin and placebo groups after 18 months of therapy.¹⁵⁷ In TECOS, eGFR declined at the same rate in both treatment groups over a 4year period but was slightly higher (1.34 ml/ min/1.73 m²) in the sitagliptin group than in the placebo group.^{158,163} This difference occurred in the first 4 months after randomisation, was similar across eGFR categories and was sustained after adjustment for baseline eGFR and HbA_{1c} levels.¹⁶³ 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.

Thus, particularly in patients with high albuminuria or low eGFR at baseline, DPP-4 inhibitors modestly reduce albuminuria in large cardiovascular outcome trials, which seems to be glucose-independent.

Tolerability and safety aspects

Incretin-based drugs are generally well tolerated. The incidence of adverse effects with DPP-4 inhibitors is similar to placebo and is lower than for other glucose-lowering drugs.⁷⁹ The incidence of adverse events with GLP-1R agonists is, however, higher than with DPP-4 inhibitors.¹⁶⁴ As glucose-lowering actions of incretin-based drugs are glucose-dependent, the risk of hypoglycaemia is very low, except when these agents are combined with insulin or sulfonylureas. 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.^{165,166} The mechanisms that underlie these gastrointestinal complaints remain uncertain, but might reflect an aversive response or reduced gastric emptying.

Acute kidney injury

Initial case reports^{11,13,167-170} and a nested case-control study in 13,504 patients with T2DM receiving DPP-4 inhibitors with <1 year of follow-up¹⁷¹ suggested that incretin-based drugs may increase AKI risk. Most patients used RAAS-inhibiting agents and diuretics,^{11,13,167-170} which, together with nausea and vomiting, might have resulted in reduced circulating volume and P_{GLO} , leading to renal failure (Chapter 4).^{11,46} Alternatively, these agents could lead to acute interstitial nephritis.¹⁷² An increased AKI risk finding was however not confirmed by a retrospective database study,¹⁷³ prospective registration studies⁷³⁻⁷⁵ or cardiovascular outcome studies.^{155,159,162} In line, we did not observe changes in UACR, NGAL or KIM-1 in T2DM patients following acute or prolonged administration with different GLP-1R agonists (Chapter 6, Chapter 7 and Chapter 8)^{28,31,32} or a DPP-4 inhibitor (Chapter 7).³¹ Notably, although preclinical and acute intervention studies in humans suggested that GLP-1R agonists inhibit the RAAS pathway, thereby potentially potentiating the effect of RAAS inhibitors, which may increase AKI risk (Chapter 4),⁴⁶ prolonged GLP-1R agonist treatment in T2DM patients does not affect circulating PRC, angiotensin-II or aldosterone (Chapter 6, Chapter 7 and Chapter 8).^{30-32,50} Conversely, in line with the renoprotective mechanisms outlined above, DPP-4 inhibitors¹⁷⁴⁻¹⁷⁹ and GLP-1R agonists^{175,180} have been reported to protect against AKI in animal models. In addition, a large prospective cohort study with 3.6 years of followup in 923,936 patients with diabetes reported reduced AKI risk in those who received DPP-4 inhibitors.¹⁸¹ However, given that GLP-1R signalling has natriuretic and diuretic properties and could increase R_A 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.

Extrarenal safety

Pancreatitis and pancreatic cancer

The putative association between incretin-based drugs, acute pancreatitis and pancreatic cancer has received much attention, but no definite causal link has been found to date.¹⁸² 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),¹⁸³ whereas DPP-4 inhibitors significantly increased the risk of acute pancreatitis relative to placebo (RR 1.79, 95% CI 1.13–2.81).¹⁸⁴ 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).¹⁸⁵ Controversy remains, however, because pancreatic cancer takes years to develop, and the duration of followup and numbers of participants in current trials are insufficient to draw definite conclusions.

Heart failure

SAVOR-TIMI 53 for saxagliptin found a significant 27% increase in hospitalisation for heart failure (particularly <1 year after randomisation) 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-analysis¹⁸⁶ 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.¹⁸⁷ 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 (Chapter 4).^{46,188} Some reassurance was provided, however, by *post hoc* analyses of SAVOR-TIMI 53¹⁸⁹ and EXAMINE,¹⁹⁰ 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 2018.¹¹⁶

Cholecystitis and gall stones

Increased risk of cholecystitis and acute gallstone disease (requiring cholecystectomy) in patients receiving liraglutide was reported in LEADER¹⁵³ and SCALE,⁸¹ but was not seen with other GLP-1R agonists or DPP-4 inhibitors. Rapid weight loss might only partly explain

Table 2. Renal outcomes in CV outcome studies of incretin-based drugs in high-risk patients with type 2 diabetes mellitus

Trial (year)	Drug	Patients (<i>n</i>)	Baseline risk factor control		
			HbA _{1c} (%)	BP (mmHg)	ACE inhibitor/ARB (%)
GLP-1 receptor agonists					
ELIXA (2015)	Lixisenatide	CV 6,068; renal 5,633	7.7	129.5/70.2	85
LEADER (2016)	Liraglutide	9,340	8.7	135.9/77.1	83
SUSTAIN-6 (2016)	Semaglutide	3,297	8.7	135.7/77.1	84
EXSCEL (2017)	Exenatide once- weekly	14,752	8.0	135.4/78.1	40
DPP-4 inhibitors					
SAVOR-TIMI 53 (2013)	Saxagliptin	CV 16,492; renal 15,760	8.0	NR	82

Follow-up	Renal end point	Exploratory renal outcomes (change from baseline placebo/intervention or HR 95% CI or prevalence placebo/intervention)	Refs
CV 25 months; renal 24 months	Change in UACR (%)	Month 24: 34/24*‡	89
3.8 years	Time to primary composite end point	HR 0.78 (0.67–0.92)*‡	153,159,161
	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)	
2.1 years	New or worsening nephropathy	HR 0.64 (0.46–0.88)*	154
	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)	
3.2 years	Microalbuminuria prevalence (%)	7.5/7.2	155
	Macroalbuminuria prevalence (%)	2.8/2.2	
	ESRD prevalence (%)	0.9/0.7	
2.1 years	Improved UACR (%)	Year 1: 9.6/11.8* Year 2: 9.2/11.1* Study end: 8.7/10.7*	156,162
	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)	
	Improved UACR (%)	Year 1: 9.6/11.8* Year 2: 9.2/11.1* Study end: 8.7/10.7*	

Table 2. (continued)

Trial (year)	Drug	Patients (n)	Baseline risk factor control		
			HbA _{1c} (%)	BP (mmHg)	ACE inhibitor/ARB (%)
EXAMINE (2013)	Alogliptin	CV 5,380; renal 839	8.0	NR	82
TECOS (2015)	Sitagliptin	CV 14,671; eGFR 13,604; UACR 3,832	7.2	135.0/77.2	79

*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 3month HbA_{1c} levels (ACR placebo +32%, lixisenatide +26%). §Results did not materially differ across HbA_{1c}-, body weight- and blood pressure-tertiles at month-6, although this was not formally tested. Results were similar when the risk of the composite renal outcome was adjusted in a time-dependent manner for the change from baseline in HbA_{1c}, body weight, and systolic blood pressure, separately and together. ¶Persistent doubling of serum creatinine level and eGFR ≤ 45 ml/min/1.73 m² using MDRD. ††Persistent doubling of serum creatinine level and eGFR < 45 ml/min/1.73 m² using MDRD. †††And/or creatinine > 6.0 mg/dl (530 μ mol/l). ††††Similar results according to baseline eGFR stage.

higher rates of gallbladder events with liraglutide, as excess risk was seen across all weight-loss categories.^{81,153} Although acute exenatide administration reduces gallbladder emptying rate in healthy individuals,¹⁹¹ we found no such effect of 12week liraglutide in patients with T2DM.¹⁹²

Retinopathy

Increased rates of retinopathy events were seen in patients with T2DM receiving semaglutide in SUSTAIN6 (HR 1.76, 95% CI 1.11–2.78),¹⁵⁴ and retinopathy rates also tended to increase with liraglutide therapy in LEADER.¹⁵³ Data from seminal T1DM¹⁹³ and, to a lesser extent, T2DM studies,¹⁹⁴ together with the finding that retinopathy events occurred early after treatment initiation in the SUSTAIN6 trial, suggest that intense and rapid glucose-lowering with semaglutide might underlie this adverse effect, rather than a drug-specific effect.¹⁵⁴ Indeed, in both the semaglutide and placebo groups, the highest rates of retinopathy events were among

Follow-up	Renal end point	Exploratory renal outcomes (change from baseline placebo/ intervention or HR 95% CI or prevalence placebo/ intervention)	Refs
18 months	Worsened UACR (%)	Year 1: 11.4/9.4* Year 2: 14.2/12.4* Study end: 15.9/13.3*	157
	Change in UACR (mg/g)	Study end: -34.3*	
	Change in UACR category	Study end: lowered by saxagliptin*	
	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	
CV 3 years; renal 4 years	Microalbuminuria (%)	Year 3: 7.9/7.8	158,163
	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)*	

Difference remained after adjustment for time from randomisation when eGFR was measured, baseline eGFR, baseline HbA_{1c}, change in HbA_{1c} and region (-1.43 ml/min/1.73 m², 95% CI -1.88 to 0.98). *Similar results according to baseline eGFR stage. ACE, angiotensin-converting enzyme; ARB, angiotensin-II receptor blocker. BP, blood pressure; CV, cardiovascular; DPP-4, dipeptidyl peptidase 4; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; 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.

patients who experienced a >1.5% drop in HbA_{1c} levels, whereas the lowest rates were among patients who experienced reductions of <0.5%.^{154,195}

Uncertainties and future perspectives

The finding in “smaller”-sized clinical studies, pooled analyses and cardiovascular outcome studies that incretin-based drugs 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. Although most trials did not properly adjust renal outcome for impact of glycaemic differences, recent additional analyses in LEADER indicated a drug-benefit for liraglutide (Chapter 12).¹⁶¹ *Post hoc* analyses of ELIXA, SUSTAIN-6 and EXSCEL are now highly anticipated to further ascertain non-glycaemic, drug-specific benefits.¹⁶⁰ Amelioration of glomerular hyperfiltration has

been suggested to partly mediate the renal benefits of incretin-based drugs. However, in our dedicated clinical studies, renal haemodynamic function in T2DM patients with normal renal function did not improve upon GLP-1R agonist or DPP-4 inhibitor treatment. Thus, indirect renoprotective effects, such as weight loss and blood pressure reductions, seem more plausible to account for improvements in renal outcome, while the relative importance of improved postprandial lipid levels or reduced systemic or tissue inflammation will likely be impossible to assess. Furthermore, 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 glimepiride¹⁹⁶) and GRADE trials (comparing liraglutide, sitagliptin, glimepiride and insulin glargine¹⁹⁷) 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 drugs improve albuminuria, but this outcome is regarded as a potential surrogate renal end point (Chapter 2),² and effects on clinically relevant renal outcomes remain uncertain. Conversely, SGLT2 inhibitors not only improve albuminuria, but also reduce established renal end points, such as doubling of serum creatinine and ESKD.^{198,199} It is therefore not surprising²⁰⁰ that the renoprotective potential of SGLT2 inhibition is subject of three ongoing dedicated renal outcome studies, in patients with DKD (CREDENCE, results are expected in 2019²⁰¹), CKD (Dapa-CKD, 2020²⁰²) and both DKD and CKD (press release²⁰³). In contrast, no ongoing studies of incretin-based drugs in atrisk 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 drugs and SGLT2 inhibitors, which improve glycaemia by inducing glycosuria,²⁰⁴ 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.²⁰⁵ The findings of the DURATION8 trial,²⁰⁶ 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 drugs and SGLT2 inhibitors in patients with T2DM also encompass blood pressure, LDL cholesterol, natriuresis and perhaps renal haemodynamics.²⁰⁷ 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

Despite improvements in the management of renal risk factors in T2DM patients, and the use of RAS inhibitors, residual renal risk remains, and novel strategies or new therapeutic options are

needed to reduce DKD-burden in this population. After more than a decade of use of incretin-based drugs, the understanding and value of therapeutically engaging GLP-1R-mediated mechanisms for T2DM treatment are becoming increasingly clear. In addition to glucose-lowering, incretin-based drugs 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 renal risk factor control in clinical practice. Furthermore, GLP-1 and associated drugs increase the renal excretion of electrolytes (most notably sodium), although GLP-1R agonist or DPP-4 inhibitor treatment does not improve glomerular hyperfiltration in T2DM patients with normal renal function. Evidence of pooled analyses, as well as results of recent 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. Thus, although the renal benefits are less pronounced than those of SGLT2 inhibitors, incretin-based drugs seem to be of added value in alleviating the worldwide DKD-burden. However, specifically designed (renoprotection) trials in high-risk T2DM patients, preferably using active comparators, would be needed to firmly establish their place in renoprotective management in this population.

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Supplemental information

Supplemental Table 1. Renal haemodynamic, tubular and RAAS-effects of incretin-based drugs

Study	Intervention	Design	Population Age (yrs); BMI (kg/m ²)	Baseline GFR / ERPF (FF)	Baseline Albuminuria
Type 2 diabetes					
Acute administration					
Gutzwiller 2004 ¹	GLP-1-peptide infusion	Cross-over, acute- infusion (3-h) vs placebo	16 M (25% T2DM); Age 45 years BMI 36.5 kg/m ² HbA _{1c} not reported	GFR (CrCl): 151 ml/min	All patients normo- albuminuria
Asmar 2016 ²	GLP-1-peptide infusion	Cross-over, acute infusion (3-h) vs placebo, single blind	8 M; age 58; BMI 28.4; HbA _{1c} 7.3%	GFR ~125.1 ml/min ERPF (⁵¹ Cr-EDTA) ~664 ml/min FF ~18.8%	All patients normo- albuminuria
Tonneijck 2016 ³ & 2018 ⁴	Exenatide- infusion	Parallel-group, acute-infusion (2.5-h) vs placebo	52 M/F; age 63; BMI 31.1; HbA _{1c} 7.3%	GFR (inulin) 82.5 ml/ min/1.73m ² ERPF (PAH) 344 ml/ min/1.73m ² FF 24.5%	14 patients UACR ≥3 mg/ mmol
Skov 2016 ⁵	Liraglutide 1.2 mg	Cross-over, acute s.c. administration (14.5-h) versus placebo	11 M; age 54; BMI 29; HbA _{1c} 6.3%	GFR (⁵¹ Cr-EDTA): 106.2 ml/min/1.73m ² MRI-based RBF: 697 ml/ min	1 patient UACR ≥3 mg/g
Prolonged intervention					
von Scholten 2015 ^{6,7}	Liraglutide 1.8 mg	Uncontrolled, open-label, (a) 7-weeks & (b) 21 days washout	31 M/F; age 64; BMI 31.9; HbA _{1c} 7.7%	GFR (⁵¹ Cr-EDTA): 99.5 ml/min/1.73m ²	13 patients UACR ≥3 mg/ mmol
von Scholten 2017 ⁸	Liraglutide 1.8 mg	Cross-over, 12-week intervention vs placebo	27 M/F; age 65; BMI 31.9; HbA _{1c} 7.8%	GFR (⁵¹ Cr-EDTA): 75 ml/ min/1.73m ² ²⁶	All patients ACR ≥3 mg/ mmol
Tonneijck 2016 ⁹ & 2018 ⁴	Liraglutide 1.8 mg Sitagliptin 100 mg	Parallel-group, 12-week intervention vs placebo's	55 M/F; age 63; BMI 31.8; HbA _{1c} 7.3%	GFR (inulin): 83.1 ml/ min/1.73m ² ERPF (PAH): 356 ml/ min/1.73m ² FF 23.7%	14 patients ACR ≥3 mg/ mmol
Ott 2016 ¹⁰	Linagliptin 5 mg	Parallel- group, 4-week intervention vs placebo's	62 M/F; age 57; BMI; 29.7; HbA _{1c} 6.9%	GFR (inulin): 140.5 ml/ min/1.73m ² ERPF (PAH): 607 ml/ min/1.73m ² FF 23.2%	Whole-group averaged normo- albuminuria

Blood pressure	Glycaemic / weight- difference	Renal haemodynamic effects	Tubular effects
Not reported	↓ PG by max. ~1.4 mmol/L	↓ GFR by 9 ml/min*	↑ sodium-, chloride-, calcium-excretion, tubular water reabsorption, pH, urine output; ↔ potassium excretion; ↓PRA
↔ SBP, DPB, MAP	Clamped glucose (~7.0-8.0 mmol/L) [§] ; difference ↓ PG by 0.8 mmol/L	↔ GFR and ERPF	↔ PRC, aldosterone, proANP, ANP
↑ DBP by 3 mmHg ↑ MAP by 6 mmHg	↓ BG by average. 1.4 mmol/L	↔ GFR, ERPF, FF, P _{GLO} , R _E ; ↑ R _A	↑ sodium-, potassium, uric acid-excretion, urine pH; ↓ urea-excretion, urinary flow; ↔ PRC
↑ SBP by 6 mmHg ↑ DBP by 3 mmHg	Clamped glucose (6-8 mmol/L) [§] ; difference ↓ BG by 0.8 mmol/L	↔ GFR and ERPF	↑ sodium-, lithium-excretion; ↔ potassium excretion, urine flow; ↓ ANGII; ↔ PRC, AGT, aldosterone, ANP, proANP
↔ SBP	↓ HbA _{1c} by 0.6% ↓ Weight by 2.5kg	(a) ↓ GFR by 10.8 ml/min (reversible) (b) ↔ GFR	(a) ↑ sodium-excretion; ↓ proANP (b) ↔ sodium-excretion, proANP
↓ SBP ↔ DBP	↓ HbA _{1c} by 1.0% ↓ Weight by 2.4kg	↔ GFR [‡]	↔ PRC, PRA, Ang-II, aldosterone
(a) ↓ SBP by 8 mmHg (b) ↓ SBP by 9 mmHg ↔ DBP, MAP	(a) ↓ HbA _{1c} 1.3%, BG 1.3 mmol/L (b) ↓ HbA _{1c} 0.8%, BG 0.7 mmol/L	↔ GFR, ERPF, FF, R _A , R _E (a) ↔ P _{GLO} , (b) ↓ P _{GLO} [%]	↔ sodium, potassium, urea, uric acid-excretion-excretion, urine pH, urinary flow, PRC [®]
Not reported	↓ HbA _{1c} ~0.2% [^] ↓ BG ~0.7 mmol/L [^]	↔ GFR, ERPF, FF, P _{GLO} , R _A , R _E	Not reported

Supplemental Table 1. (continued)

Study	Intervention	Design	Population		Baseline	
			Age (yrs); BMI (kg/m ²)	Baseline GFR / ERPF (FF)	Albuminuria	
Tonneijck 2017 ¹¹ & 2018 ^{4,12}	Lixisenatide 20 µg	Parallel-group, 8-week intervention vs titrated insulin glulisine	35 M/F; age 62; BMI 31.5; HbA _{1c} 8.0%	PP-GFR (inulin): 86.8 ml/min/1.73m ² PP-ERPF (PAH): 331 ml/min/1.73m ² PP-FF 26.5%	8 patients ACR ≥3 mg/mmol	
Non-diabetes						
Acute intervention						
Skov 2013 ¹³	GLP-1 peptide infusion	Cross-over, acute-infusion (2-h) vs placebo	12 M; age 23; BMI 23.0	GFR (⁵¹ Cr-EDTA): 123.8 ml/min ERPF (¹²³ I-hippuran): 598 ml/min FF 20.9%	Normo-albuminuria	
Asmar 2015 ¹⁵	GLP-1 peptide infusion	Cross-over, acute-infusion (2-h) vs placebo, single blind	7 M; age 47; BMI 24.0	GFR ~115.5 ml/min ERPF (Fick's Principle ⁵¹ Cr-EDTA) ~716 ml/min FF ~16.1%	Not reported	
Muskiet 2016 ¹⁶	Exenatide-infusion	Parallel-group, acute-infusion (2.5-h) vs placebo	10 M; age 22; BMI 29.4	GFR (inulin): 97 ml/min/1.73m ² ERPF (PAH): 501 ml/min/1.73m ² FF 19.8%	All patients normo-albuminuria	

All trials are randomised and double-blind unless stated otherwise. Baseline renal haemodynamics in cross-over studies is measured during placebo-infusion. *Statistically significant. ⁴Baseline GFR was GFR measured after 12-weeks placebo-intervention. ⁸Clamped by infusion of either glucose 20% or small doses of rapid-acting insulin if glucose concentrations were below or above this range, respectively. ⁵Clamped by infusion of glucose in all but the first studied patient (in which arterial glucose decreased by ~4 mmol/L, with no symptoms of hypoglycaemia or increased arterial plasma adrenaline). ⁹In the 2 patients with hyperfiltration (GFR > 135 mL/min/1.73 m²) included in this study, GFR was reduced by >30% during liraglutide treatment. ⁶After 2 weeks, an increase in fractional sodium and urea-excretion was observed with sitagliptin. At week-12, fractional urea-excretion was not changed with sitagliptin in the fasting state, but was increased during the renal tests. ⁷Partly explained by

Blood pressure	Glycaemic / weight- difference	Renal haemodynamic effects	Tubular effects
↑ SBP, DBP, MAP by 9 mmHg	↔ HbA _{1c} ↓ BG 1.8 mmol/L	↔ GFR, ERPF, FF, P _{GLO} , R _A , R _E	↑ sodium-, chloride-, potassium-excretion, urine pH; ↓ magnesium-, calcium-, phosphate-excretion ↔ uric acid-excretion, urine flow, free water clearance, PRC, ANG-II, aldosterone
↔	↓ PG by ~0.6 mmol/L	↔ GFR, ERPF, FF	↑ sodium-, calcium-, lithium-excretion; ↔ potassium excretion, urine flow, proANP ¹⁴ ↓ Ang-II
↑ SBP by 5 mmHg ↑ PP by 5 mmHg	↓ AG by max. ~1.2 mmol	↔ GFR and ERPF	↔ Na, K, H ⁺ , lithium-excretion, urine output, ANP, proANP; ↓ PRC
↔ SBP, DBP, MAP	↓ PG by 0.5 mmol/L	↑ GFR, ERPF and P _{GLO} ; ↓ R _A ; ↔ R _E ;	↑ sodium-, uric acid ¹⁴ -excretion, urine pH; ↔ potassium-excretion

the reduction of plasma protein, which is not adapted in the Gomez estimation used for this parameter. ^No significance level is given for between-group difference. **Abbreviations:** AG, arterial glucose; Ang-II, angiotensin-II; BG, blood glucose; BMI, body mass index; DBP, diastolic blood pressure; EDTA, ethylenediaminetetraacetic acid; ERPF, effective renal plasma flow; F, female; FF, filtration fraction; GFR, glomerular filtration rate; M, male; MAP, mean arterial pressure; PG, plasma glucose; PP, postprandial; PRA, plasma renin activity; PRC, plasma renin concentration; proANP, pro-atrial natriuretic peptide; R_A, afferent renal arteriolar resistance; RBF, renal blood flow; R_E, efferent renal arteriolar resistance; SBP, systolic blood pressure; UACR, urinary albumin/creatinine ratio.

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Supplemental Table 2. Effects of GLP-1 receptor agonists and DPP-4 inhibitors on renal outcome in preclinical models of diabetes

Study	Animal Model	Drug (duration)	Renal effects
GLP-1 receptor agonists			
Sancar-Bas (2015) ¹	STZ diabetic BALB/c mice	Exendin-4 (30 days)	M IL-1 β ↓, TNF- α ↓, MCP-1 ↓, ICAM-1 ↓, TGF- β 1 ↓, renal ROS production ↓, BG ↓
Takashima (2016) ²	KK/Ta-Akita mice	Liraglutide (6 wks)	H Histological renal changes (including tubular epithelial cell proliferation) ↓
			P Glomerular hyperfiltration ↔, Urinary sodium and potassium ↔
			M Albuminuria ↓, Renal oxidative stress markers ↓, Renal fibrogenic markers, BG ↓
			H Glomerulosclerosis ↓, Periglomerular fibrosis ↓, Mesangial expansion ↓, Podocyte loss ↓,
Fujita (2014) ³	KK/Ta-Akita mice	Liraglutide (4 wks)	P Glomerular hyperfiltration ↓
			M Albuminuria ↓, glomerular superoxide and NAD(P)H oxidase ↓, renal cAMP and PKA activity ↑, BG ↔
Rieg (2012) ⁴	C57BL/6 (wild type) mice, GLP-1R k.o. and diabetic db/db mice	Exendin-4 (3 h)	H Kidney weight ↓, Mesangial matrix expansion ↓, Thickening of GBM ↓, Fibronectin accumulation glomerular capillary walls ↓, Podocyte number ↑
			P GFR ↑, diuresis ↑, FE-sodium ↑ and NHE3-expression ↑ in WT but not in GLP-1R k.o mice. In db/db mice: Diuresis ↑ and FE-sodium ↑
Park (2007) ⁵	db/db mice	Exendin-4 (8 wks)	P Glomerular hyperfiltration ↓
			M Albuminuria ↓, BG/HbA _{1c} ↔
			H Glomerular hypertrophy ↓, Mesangial matrix expansion ↓, TGF- β 1 expression ↓, Type IV collagen accumulation and associated glomerular lipid accumulation ↓, Inflammatory cell infiltration in glomeruli ↓, Apoptotic glomerular cells in glomeruli ↓
Hirata (2009) ⁶	Salt-sensitive obese db/db mice	Exendin-4 (12 wks)	P 1) FE-sodium ↑
			P 2) Angiotensin-II induced hypertension ↓
	Angiotensin-II-infused C57BLK6/J mice		

Supplemental Table 2. (continued)

Study	Animal Model	Drug (duration)	Renal effects
Zhou (2014) ⁷	STZ diabetic Wistar rats	Liraglutide (12 wks)	P Serum creatinine and urea ↓ Albuminuria ↓, NF-κB ↓, TNF-α ↓, IFN-γ, IL-6 ↓, MCP-1 ↓, BG ↔ H Inflammatory cell infiltration ↓, Mesangial expansion ↓, Proliferation in glomeruli ↓
Ojima (2013) ⁸	STZ diabetic Wistar rats	Exendin-4 (2 wks)	M Albuminuria ↓, AGE-induced RAGE expression ↓, ROS and ADMA generation in tubular cells ↓, Urinary 8-OHdG excretion ↓ H Histopathologic renal changes ↓ P Glomerular hyperfiltration ↓
Kodera (2011) ⁹	STZ diabetic Sprague-Dawley rats	Exendin-4 (8 wks)	M Albuminuria ↓, ICAM-1/Collagen type IV levels ↓, Oxidative stress markers ↓, NF-κB ↓, BG ↔ H Glomerular hypertrophy ↓, Mesangial matrix expansion ↓, Macrophage infiltration ↓ M Albuminuria ↓, Urinary 8-OHdG excretion ↓, Urinary MDA excretion ↓, Renal oxidative stress markers ↓ (TGF-β1 / Fibronectin), BG ↔
Hendarto (2012) ¹⁰	STZ diabetic Wistar rats	Liraglutide (4 wks)	M Albuminuria ↓, Urinary 8-OHdG excretion ↓, Urinary MDA excretion ↓, Renal oxidative stress markers ↓ (TGF-β1 / Fibronectin), BG ↔
DPP-4 inhibitors			
Takashima (2016) ²	KK/Ta-Akita mice	Linagliptin (6 wks)	P Glomerular hyperfiltration ↓ urinary sodium and potassium ↑ (effects blunted by SDF-1 blockade) M Albuminuria ↓, Renal oxidative stress markers ↓, Renal fibrogenic markers, Renal SDF-1-α ↑, BG ↓ H Glomerulosclerosis ↓, Periglomerular fibrosis ↓, Mesangial expansion ↓, Podocyte loss ↓, M Albuminuria ↓, TGF-β1 ↓, BG ↔
Jung (2016) ¹¹	STZ diabetic C57BL/6J mice	Gemigliptin (8 wks)	M Albuminuria ↓, TGF-β1 ↓, BG ↔
Alter 2012 ¹²	STZ diabetic eNOS k.o C57BL/6J mice	Linagliptin (12 wks)	H Thickening of GBM ↓, Tubular atrophy and renal fibrosis ↓ M Albuminuria ↓ only when combined with telmisartan, TNF-α ↓, BG ↔ H Glomerulosclerosis ↓

Supplemental Table 2. (continued)

Study	Animal Model	Drug (duration)	Renal effects
Gangadharan Komala (2016) ¹³	STZ diabetic eNOS k.o. mice	Saxagliptin (12 wks)	Albuminuria ↔, Fibronectin ↓, NF-κB ↓, Macrophage marker ↓, BG ↔
Kanasaki (2014) ¹⁴	STZ diabetic CD-1 mice	Linagliptin (4 wks)	Glomerular hypertrophy ↓, Glomerulosclerosis ↔, Tubulointerstitial fibrosis ↓ Albuminuria ↓, TGF-β1 ↓, TGF-β2 ↓, BG ↔ Blood pressure, body weight en BG niet anders
Shi (2015) ¹⁵	STZ diabetic CD-1 mice	Linagliptin (20 wks)	Glomerular hypertrophy ↓, Mesangial matrix expansion ↓, Interstitial fibrosis ↓, Kidney weight ↓, Cells undergoing EdnMT ↓, Profibrotic genes ↓, microRNA 29s suppression ↓ Plasma cystatin C ↓
Tsavidaridis (2015) ¹⁶	db/db mice	Sitagliptin (32 wks)	Integrin β1, phospho-integrin β1, TGF-β1, and TGF-β2 expression ↓, BG ↔ Glomerular hypertrophy ↓, Mesangial matrix expansion ↓, interstitial fibrosis ↓, Cells undergoing EdnMT ↓ (suggested) [niet specifiek benoemd] Serum creatinine and urea ↓
Jung (2015) ¹⁷	db/db mice	Gemigliptin (12 wks)	Albuminuria ↓, hsCRP, BG ↓ Albuminuria ↓, Urinary nephirin ↓, Urinary 8-OHdG ↓, renal AOPP, renal RAGE ↓ BG/HbA _{1c} ↔
Sharkovska (2014) ¹⁸	db/db mice	Linagliptin (12 wks)	Glomerulosclerosis ↓, Mesangial matrix expansion ↓, Podocyte apoptosis ↓, Albuminuria ↓ compared to enalapril-treatment, BG ↔
Rieg (2012) ⁴	C57BL/6 (wild-type) mice GLP-IR k.o. db/db mice	Alogliptin (3 h)	Markers of glomerulosclerosis and tubulointerstitial fibrosis ↓, Podocalyxin ↑ Diuresis ↑ and FE-sodium ↑ in both WT and GLP-IR.k.o mice, no effect in db/db mice
Nakashima (2014) ¹⁹	STZ diabetic Sprague-Dawley rats	Linagliptin (2 wks)	Albuminuria ↓, renal AGEs ↓, renal RAGE ↓, renal 8-OHdG ↓, renal ICAM-1 ↓, BG ↔
Liu (2012) ²⁰	STZ diabetic Sprague-Dawley rats	Vildagliptin (24 wks)	Lymphocyte infiltration ↓ Creatinine clearance ↑

Supplemental Table 2. (continued)

Study	Animal Model	Drug (duration)	Renal effects
			M Albuminuria ↓, TGF-β1 expression ↓, Urinary cAMP excretion ↑, Urinary 8-OHdG excretion ↓, BG ↔
Kodera (2014)²¹	STZ diabetic Sprague-Dawley rats	PKF275-055 (8 wks)	H Interstitial expansion ↓, Glomerulosclerosis ↓, Thickening of GBM ↓ M Albuminuria ↓, Macrophage infiltration ↓, Inflammatory markers ↓, HbA _{1c} ↔
Sharma (2014)²²	NAD-STZ diabetic Wistar rats	1) Vildagliptin (5wks) 2) Vildagliptin + telmisartan (5 wks)	H Histological renal changes ↓ P 1 + 2) serum creatinine ↓
			M 1 + 2) Albuminuria ↓, BG ↓
			H 2 > 1) Glomerulosclerosis ↓, Interstitial fibrosis Mesangial matrix expansion ↓, Nodular lesions ↓, Thickening of GBM ↓
Vaghasiya (2011)²³	NAD-STZ diabetic Wistar rats undergoing ischemia/reperfusion	Sitagliptin (2 wks)	M Lipid peroxidation ↓, xanthine oxidase activity ↓, MPO activity ↓, nitric oxide levels ↓, glutathione ↑, glutathione peroxidase ↑, SOD ↑, catalase ↑, BG ↓
Mega 2011²⁴	ZDF rats	Sitagliptin (6 wks)	H Apoptosis ↓, preservation of renal morphology P No change in creatinine and urea levels M Lipid peroxidation ↓, BG/HbA _{1c} ↓ H Glomerular lesions ↓ (fibrosis, sclerosis, mesangial expansion, atrophy, basement membrane atrophy), Tubulointerstitial lesions ↓ (hyaline cylinders, tubular degeneration, basement membrane irregularity), Vascular lesions ↓ (hyalinosis)
Marques (2014)²⁵	ZDF rats	Sitagliptin (6 wks)	M IL-1β ↓, TNF-α ↓, BG/HbA _{1c} ↓ H Glomerular lesions ↓, Tubulointerstitial ↓, Apoptosis ↓ M 1 + 2) IL-1β ↓, IL-13 ↓, BG/HbA _{1c} ↔
Wang (2012)²⁶	ZDF rats	1) Vildagliptin (15 wks) 2) NWT-03 (both ACEi and DPP-4i) (15 wks)	M 2) Albuminuria ↓, TNF-α ↓

Supplemental Table 2. (continued)

Study	Animal Model	Drug (duration)	Renal effects
Tofovic (2010) ²⁷	ZSF1 rats (lean vs obese)	Sitagliptin	H 1 + 2) glomerulosclerosis ↓
Nistala (2014) ²⁸	ZO rats	Linagliptin (8 wks)	P Angiotensin-II induced renal vasoconstriction ↑ M Albuminuria ↓, plasma NADPH oxidase activity ↓, renal ROS production ↓, plasma SDF-1-α ↑ H Glomerular hypertrophy ↓, Glomerulomegaly ↓, Podocyte hypertrophy ↓, Ultrastructural changes filtration barrier ↓

8-OHdG, 8-oxo-2'-deoxyguanosine; ACE-I, angiotensin-converting-enzyme inhibitor; ADAM, asymmetric dimethylarginine; AGEs, advanced glycation end products; AOPP, advanced oxidation protein products; BG, blood glucose; cAMP, cyclic adenosine monophosphate; EdnMT, endothelial-to-mesenchymal transition; eNOS, endothelial nitric oxide synthase; FE, fractional excretion; H, histology; hsCRP, high-sensitivity c-reactive protein; ICAM, intercellular adhesion molecule; IFN, interferon; IL, interleukin; M, biomarkers; MCP, monocyte chemotactic protein; MPO, myeloperoxidase; NAD, nicotinamide; NF-κB, nuclear factor kappa B; P, physiology; ROS, reactive oxygen species; STZ, streptozotocin; TGF, transforming growth factor; TNF, tumour necrosis factor; ZDF, Zucker diabetic fatty; ZO, Zucker obese.

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