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Postprandial renal haemodynamic effect of lixisenatide vs once-daily insulin-glulisine in patients with type 2 diabetes on insulin-glargine: an 8-week, randomised, open-label trial

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Abstract

Aims

To determine whether lixisenatide, a prandial short-acting glucagon-like peptide receptor agonist (GLP-1RA), ameliorates postprandial glomerular hyperfiltration in patients with type 2 diabetes mellitus (T2DM) compared with insulin-glulisine (iGlu).

Methods

Postprandial renal haemodynamic effects of 8-week treatment with lixisenatide 20 µg vs once-daily titrated iGlu were measured in 35 overweight patients with T2DM inadequately controlled on insulin-glargine, with or without metformin [mean ± SD age 62 ± 7 years, HbA_{1c} 8.0% ± 0.9%, estimated glomerular filtration rate (GFR) 85 ± 12 mL/min/1.73 m^2, median (IQR) urinary albumin/creatinine ratio 1.5 (0.9-3.0) mg/mmol]. After a standardised breakfast, GFR (primary endpoint) and effective renal plasma flow (ERPF) were determined by inulin and para-aminohippuric acid renal clearance, respectively, based on timed urine sampling. Intrarenal haemodynamic functions were estimated using Gomez equations.

Results

Compared with iGlu, lixisenatide did not affect GFR [+0.1 mL/min/1.73 m^2 (95% CI −9 to 9)], ERPF [−17 mL/min/1.73 m^2 (−61 to 26)], other (intra-)renal haemodynamics or renal damage markers, but increased fractional sodium excretion [+0.25% (0.09-0.41)] and urinary pH [+0.7 (0.3-1.2)]. Plasma renin, angiotensin-II and aldosterone were unchanged. Lixisenatide and iGlu reduced HbA_{1c} similarly, by 0.8% ± 0.1% and 0.6% ± 0.1%, respectively, while postprandial glucose was lower with lixisenatide (p=0.002). Compared with iGlu, lixisenatide reduced bodyweight [−1.4 kg (−2.5 to −0.2)] and increased postprandial mean arterial pressure [+9 mm Hg (4-14)].

Conclusion

Eight-week lixisenatide treatment does not affect postprandial (intra-)renal haemodynamics compared with iGlu when added to insulin-glargine in patients with T2DM without overt nephropathy. Prolonged lixisenatide treatment has a sustained natriuretic effect, which is in contrast to previous reports on long-acting GLP-1RA, reduces body weight and increases postprandial blood pressure compared with iGlu.

Introduction

Glucagon-like peptide-1 (GLP-1) receptor agonists (RA) are injectable drugs for the management of hyperglycaemia in patients with type 2 diabetes (T2DM). GLP-1RAs classically lower glucose concentrations by stimulating pancreatic insulin secretion and suppressing glucagon output in a glucose-dependent manner.[1] In addition to glycaemic control, GLP-1RAs also benefit other T2DM-related renal risk factors, including obesity, hypertension and dyslipidaemia.[2,3] As such, GLP-1RAs could mitigate the morbidity, mortality and (psycho)social burden associated with diabetic kidney disease.

Glomerular hyperfiltration is considered an independent renal risk factor in T2DM. [4] Hyperglycaemia, dysregulated vasoactive factors (e.g. nitric oxide and angiotensin-II) and impaired tubuloglomerular feedback (TGF) signals are proposed to be involved in the pathophysiology of hyperfiltration and augmented glomerular hydraulic pressure (P_{GLO}) in these patients.[4] Upon meal ingestion, several vasoactive factors are released or biologically activated, while absorbed glucose and amino acids directly and indirectly (e.g. via glucagon) increase tubular sodium reabsorption proximal to the macula densa, thereby inhibiting TGF leading to vasodilation of the afferent renal arteriole.[4,5] Indeed, a physiological increase in glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) is commonly observed following a meal in humans,[6] but is assumed to cause damage to the kidney in the setting of diabetes.[4,5,7] As such, pharmacological blunting of this response in T2DM may reduce renal risk and improve long-term outcome.

Lixisenatide is a once-daily short-acting GLP-1RA that blunts postprandial glucose excursions, predominantly via delayed gastric emptying and suppression of glucagon. Addition of a short-acting GLP-1RA to basal insulin in patients with T2DM has recently gained clinical interest due to their favourable effect on body weight and fewer hypoglycaemic events compared with drug-intensification with prandial insulin.[8] Interestingly, lixisenatide has recently been associated with reduced progression of urinary albumin excretion in the landmark ELIXA (Evaluation of LIXisenatide in Acute Coronary Syndrome) trial, which could be regarded partially independent of glycaemic control.[9] Some mechanistic studies in humans suggested that this may relate to improvements in (intra-)renal haemodynamics in the fasting state,[10-13] although others did not find evidence to support this.[14-17] Effects in the postprandial "stimulated" state, in which many humans spend most of their day, have not been determined. Therefore, we aimed to explore the effects of lixisenatide on the renal system in the postprandial state – when the drug has its most pronounced actions. To explore glucose-independent properties and allow for clinically relevant comparisons, prandial insulin-glulisine (iGlu; once-daily) was selected as an active control. We hypothesised that, in addition to improving other renal risk factors,[9] lixisenatide reduces postprandial GFR, ERPF and P_{GLO} in patients with T2DM by slowing gastric empting rate, activating TGF and suppressing the activity of the renin-angiotensin-aldosterone system (RAAS).

Materials and methods

Trial Design

This was a phase-4, monocentre, randomised, open-label, comparator-controlled, parallel-group, intervention trial. The study consisted of a 4-week run-in period, in which anti-hyperglycaemic treatment was not altered, followed by an 8-week intervention period.

Study population

Patients were recruited by advertisements in local newspapers. Eligible patients were Caucasian, men or post-menopausal women, aged 35 to 75 years, who were diagnosed with T2DM with an HbA_{1c} of 6.5% to 10.0% (48-86 mmol/mol) and a body mass index >25 kg/m^2. Patients were treated with basal insulin-glargine (stable dose ±20% for ≥3 months, injection in the evening), with or without metformin (stable dose for ≥3 months). Use of other antihyperglycaemic agents was not allowed. Fasting plasma glucose needed to be below 10 mmol/L unless the glargine dose exceeded >50 units. Blood pressure was under control (i.e. <140/90 mm Hg); in case of previously diagnosed hypertension and/or albuminuria treatment included at least a stable dose of a renin-angiotensin system (RAS) inhibitor for ≥3 months. Exclusion criteria included history of pancreatic, active liver or malignant disease (excluding basal cell carcinoma), estimated GFR <60 mL/min/1.73 m^2, urinary retention (bladder ultrasonography at screening visit was performed to objectively assess bladder emptying), current urinary tract infection, active nephritis, or use of diuretics that could not be stopped 3 months prior to and during the intervention period. Written informed consent was obtained from all patients before any trial-related activities. The study protocol, protocol amendments and any other protocol-specific documents were reviewed and approved by local authorities and the ethics review board of the VU University Medical Center (Amsterdam, The Netherlands). The study complied with the Declaration of Helsinki and Good Clinical Practice guidelines, and was registered at ClinicalTrials.gov (ID: NCT02276196).

Randomisation and intervention

Patients were randomised to lixisenatide or iGlu in a 1:1 ratio (with a block-size of 4, performed by an independent trial pharmacist using computer-generated numbers). Patients were randomised to receive lixisenatide (Sanofi Aventis, Paris, France) administered 10 µg once-daily for 2 weeks, followed by the lixisenatide 20 µg once-daily maintenance dose for the remainder of the study, injected subcutaneously ~30 min before breakfast. To limit the risk of hypoglycaemia, patients with an HbA_{1c} ≥6.5% and ≤7.5% at screening initially reduced insulin-glargine dose by 20% on the day before lixisenatide treatment initiation, which was progressively increased at the investigator's discretion to the initial dosage after 3 weeks. Patients randomised to receive iGlu (Sanofi Aventis, Frankfurt am Main, Germany) started with 2 units/d once-daily, injected <15 min before breakfast, which was self-titrated to the 2-hour postprandial glucose target ≥5.0 and ≤8.0 mmol/L, as described.[18] All patients received a self-monitoring blood glucose device for the duration of the study (GlucoMen LX Plus, Menarini Diagnostics, Valkenswaard, The Netherlands).

Outcome measures

The primary endpoint was lixisenatide-induced change in GFR and ERPF from baseline to week 8, compared with iGlu, as derived from inulin and para-aminohippuric acid (PAH) clearance methodology, respectively, based on timed urine sampling. All other (intra-)renal haemodynamic variables, tubular handling of sodium and hydrogen (assessed as urinary pH), urinary flow and markers of renal damage, and other measurements obtained at baseline and after 8 weeks of treatment were considered secondary endpoints.

Concise study protocol

After an overnight fast, blood and urine were obtained for fasting outcome variables. Then, the renal tests commenced by infusing inulin and PAH. At 35 min, patients consumed a standardised mixed breakfast, together with 1.5 g of acetaminophen in suspension form.[19] During the renal testing day at week 8, patients administered lixisenatide or iGlu 30 and 10 minutes before breakfast, respectively. From 100 minutes, urine was collected by spontaneous voiding for two 45-minute periods. Blood and urine samples were taken during and after the renal tests to obtain postprandial outcome variables. Systolic blood pressure (SBP), mean arterial pressure (MAP), diastolic blood pressure (DBP) and heart rate were determined before the renal tests and drug administration, and at 120 minutes. The full study protocol, assays and calculations of renal physiology and markers of renal damage are described in the Supplemental methods.

Sample-size calculation

Although GFR and ERPF were co-primary endpoints, we based our sample size calculation on GFR, as no trials evaluated effects of GLP-1RA on ERPF at the time of study design. We calculated that 16 patients per treatment-arm would be sufficient to detect a change of 12% in GFR, assuming a standard deviation of 10 mL/min, α=0.05 and power (1 – β) of 80%.[10] To allow for a dropout rate of 15% and account for technical failures, we aimed to include 20 patients per treatment-arm.

Data management and statistics

Data were double-entered in an electronic data management system (OpenClinica LLC, version 3.6, Waltham, MA) and transferred to the final study database. Before deblinding, inulin-extraction ratios were inspected, and urine collection periods characterised by profound collection errors were discarded from the analyses. This led to exclusion of one patient randomised to iGlu. Statistical analyses were performed in the per protocol population using SPSS 22.0 (IBM SPSS, Chicago, IL), and were done according to a statistical analysis plan agreed before inspection of the data. Multivariable linear regression models were used to examine lixisenatide-induced effects compared with iGlu. Corresponding baseline values were added as independent variables, to correct for potential between-group baseline differences. To assess potential effect modification on GFR, ERPF and filtration fraction (FF), we performed subgroup analyses (which were not pre-specified, but in line with our a priori hypothesis):[20] patients with

baseline inulin-based fractional sodium excretion (FE_{Na}) or urinary albumin/creatinine ratio (UACR) of greater or less than the median of our study population and use of RAS inhibition. Within-group comparisons were analysed using paired t-tests (Gaussian distributed data) or Wilcoxon signed rank tests (non-Gaussian distributed data; also after log or square root transformation). Correlations between changes in (intra-)renal haemodynamic and predefined variables that could theoretically be of influence were assessed by Pearson's correlation or Spearman signed rank test, as appropriate. To compare between-group differences in adverse events, Fisher's exact test was used for proportions and Mann–Whitney test for total number of events. Statistical significance was considered at a 2-sided α-level of <0.05. Data are presented as mean ± SEM, median (IQR) or mean difference with a 2-sided 95% confidence interval (CI).

Results

Between September 2014 and April 2016, 51 patients were screened, of whom 40 were included, and 39 were randomised to 8-week treatment with iGlu (N=19) or lixisenatide (N=20; Supplemental Figure 1). One included patient withdrew consent before randomisation. Malaise caused one patient randomised to lixisenatide to discontinue treatment prematurely, and one patient to complete the intervention period using lixisenatide 10 µg once-daily (as 20 µg was intolerable). Two patients randomised to lixisenatide were excluded due to drug-unrelated adverse effects (i.e. intolerance to inulin/PAH and intercurrent viral infection). Overall, demographic and clinical characteristics, as well as renal risk factors at baseline, were well balanced between treatment groups (Table 1). Most participants received other treatments in addition to insulin-glargine at baseline, most commonly metformin (91%), RAS inhibitors (63%) and statins (60%); no drugs were started during the treatment period. After 8 weeks, median (IQR) iGlu dose was 17 (8-29) units/d.

Glycaemia

After 8 weeks, mean changes in HbA_{1c} from baseline were 0.8% ± 0.1% with lixisenatide (P<0.001) and 0.6% ± 0.1% with iGlu (p=0.001); the difference between treatment groups was non-significant (p=0.897), meeting the objective of glycaemic equipoise. Within- and between-group change from baseline to week 8 in fasting plasma glucose was minimal and non-significant (Table 2). Compared with iGlu, lixisenatide decreased postprandial time-averaged glucose during the renal tests [−1.8 mmol/L (95% CI −2.9 to −0.7), all values ≥4.6 mmol/L]. Lixisenatide, as compared with iGlu, lowered time-averaged insulin (p=0.057), glucagon concentrations (p=0.005) and gastric emptying rate [acetaminophen net area under the curve (AUC_{net}) and maximal concentration (p<0.001); Figure 1 and Supplemental Figure 2; Table 2]. There were no between-group differences in time-averaged C-peptide concentrations after 8 weeks of treatment.

(Intra-)renal haemodynamics

Patients randomised to lixisenatide tended to have a higher baseline postprandial GFR (p=0.064) and fasting estimated (e)GFR-modification of diet in renal disease (MDRD; p=0.052), whereas

postprandial ERPF and renal blood flow (RBF) were higher in the lixisenatide arm at baseline (p=0.009 and p=0.008, respectively); no between-group differences in other baseline (intra-) renal haemodynamic characteristics were seen before treatment. Lixisenatide, as compared with iGlu and relative to baseline, did not affect postprandial GFR [+0.1 mL/min/1.73 m^2 (95% CI −9 to 9)], ERPF [−17 mL/min/1.73 m^2 (−61 to 26)], renal blood flow or FF after 8 weeks of treatment (Figure 2 and Supplemental Figure 3; Supplemental Table 1). No treatment-induced between-group differences were observed in P_{GLO}, afferent and efferent renal arteriolar resistance (R_A and R_E, respectively) or renal vascular resistance (Figure 2; Supplemental Table 1). iGlu increased postprandial ERPF and reduced FF and R_E from week 0 to week 8 (p<0.05). A similar pattern was observed with lixisenatide, although this was non-significant (p>0.05; Figure 2). Lixisenatide increased R_A by 19% (p=0.041) from baseline to week 8, although this did not reach significance compared with iGlu. The use of absolute GFR and ERPF values that were not

Table 1. Baseline characteristics

Variables	Insulin glulisine (N=18)	Lixisenatide (N=17)
Age, years	61 ± 7	62 ± 7
Male, *n* (%)	11 (61)	12 (71)
Current smoker, *n* (%)	4 (22)	3 (18)
Bodyweight, kg	95.8 ± 17.7	99.2 ± 15.2
Body mass index, kg/m^2	31.5 ± 4.1	31.4 ± 4.1
Diabetes duration, years	14 ± 8	11 ± 5
Insulin use duration, years	5 [2-11]	5 [2-8]
Insulin dose, units	39 [32-51]	44 [41-53]
HbA_{1c}, %	7.8 ± 0.8	8.3 ± 0.8
HbA_{1c}, mmol/mol	62 ± 9	67 ± 9
Fasting plasma glucose, mmol/L	7.3 ± 2.1	6.7 ± 1.8
eGFR (CKD-EPI 2009), mL/min/1.73m^2	82 ± 12	89 ± 11
eGFR (MDRD), mL/min/1.73m^2	78 ± 12	87 ± 14
UACR, mg/mmol*	1.53 [1.24-3.17]	0.94 [0.42-2.96]
Systolic blood pressure, mmHg	138.1 ± 17.7	129.7 ± 12.9
Mean arterial pressure, mmHg	99.8 ± 14.0	94.7 ± 9.6
Diastolic blood pressure, mmHg	76.8 ± 9.9	75.0 ± 8.9
Heart rate, beats/minute	68 ± 10	65 ± 10
Metformin use, *n* (%)	16 (89)	16 (94)
Statin use, *n* (%)	11 (61)	10 (59)
Antihypertensive medication use, *n* (%)	11 (61)	11 (65)
RAS inhibitor use, *n* (%)	11 (61)	11 (65)
ACE inhibitor use, *n* (%)	6 (33)	8 (47)
ARB use, *n* (%)	5 (28)	3 (18)

Data are mean ± SD or median [IQR], unless stated otherwise. There were no significant between-group differences using appropriate tests. *27 patients had UACR <3 mg/mmol. In 8 patients UACR was ≥3 mg/mmol (5 randomised to insulin glulisine and 3 to lixisenatide). Abbreviations: ACE=angiotensin-converting enzyme. ARB=angiotensin-II receptor blocker. CKD-EPI=chronic kidney disease epidemiology collaboration. eGFR=estimated glomerular filtration rate. HbA_{1c}=glycated haemoglobin. MDRD=modification of diet in renal disease. RAS=renin-angiotensin-system. UARC=urinary albumin/creatinine ratio.

indexed for body surface area did not change the primary results (Supplemental Figure 4). There were no changes in fasting estimated GFR (Supplemental Table 1).

Renal damage markers, tubular functions and RAAS

In the iGlu arm, three patients regressed and four progressed in the fasting UACR-KDIGO category, while one patient on lixisenatide progressed in the UACR-KDIGO category. Postprandial UACR, neutrophil gelatinase-associated lipocalin/creatinine ratio (CR) or kidney injury molecule-1/CR did not change within- or between-group ($p>0.05$; Table 3). In the postprandial state, 8-week lixisenatide increased creatinine-based FE_{Na} [0.25% (0.09-0.41)], urinary pH [0.7 (0.3-1.2)] and plasma sodium ($p<0.05$), compared with iGlu and relative to baseline. Lixisenatide tended to increase absolute urinary sodium excretion within-group ($p=0.051$). Compared with iGlu, lixisenatide tended to decrease postprandial plasma renin concentrations (PRC; $p=0.052$), without affecting plasma angiotensin-II or aldosterone concentrations (Table 3).

Blood pressure and heart rate

At week 8 and before the renal tests, there were no between-group differences in fasting SBP, MAP or DBP (Figure 3; Supplemental Table 2). After drug administration, postprandial SBP, MAP and DBP all increased by 9 mm Hg with lixisenatide, compared with iGlu and relative to baseline ($p<0.05$ for all). No within- or between-group changes in heart rate were observed at any time-point after 8 weeks of treatment (Supplemental Table 2).

Bodyweight, body composition and fasting lipids

Compared with iGlu, lixisenatide reduced body weight by 1.4 kg (95% CI −2.5 to −0.2). There were no within- or between-group differences in body fat percentage, waist or hip circumference or waist/hip ratio after 8 weeks of treatment, except for a within-group decrease in waist circumference with iGlu (Table 2). Compared with iGlu, lixisenatide tended to decrease fasting high-density lipoprotein cholesterol ($p=0.068$; Table 2). Lixisenatide, as compared with iGlu, tended to reduce postprandial body water percentage ($p=0.058$). No within- or between-group changes in postprandial haematocrit were observed (Table 2).

Additional sub-group and correlation analyses

Stratification according to inulin-based FE_{Na} (median 0.84%), fasting UACR (median 1.52 mg/mmol) or use of RAS-inhibition did not reveal notable lixisenatide-induced effects on GFR, ERPF or FF in any subgroup (data not shown). In a regression model, the increase in creatinine-based FE_{Na} with lixisenatide compared to iGlu was partly explained by treatment-induced differences in postprandial MAP [the regression coefficient declined from 0.25% ($p=0.001$) to 0.21% ($p=0.042$)]. In addition, within-group changes after 8 weeks (Δ) in (intra-)renal haemodynamics were explored. ΔERPF, ΔFF or ΔR_E correlated with Δtime-averaged glucose (Supplemental Figure 5), but not with Δangiotensin-II concentrations or Δacetaminophen AUC_{net} ($p>0.05$) (data not shown). ΔR_A did not correlate with Δcreatinine-based FE_{Na} ($p>0.05$) (Supplemental Figure 5).

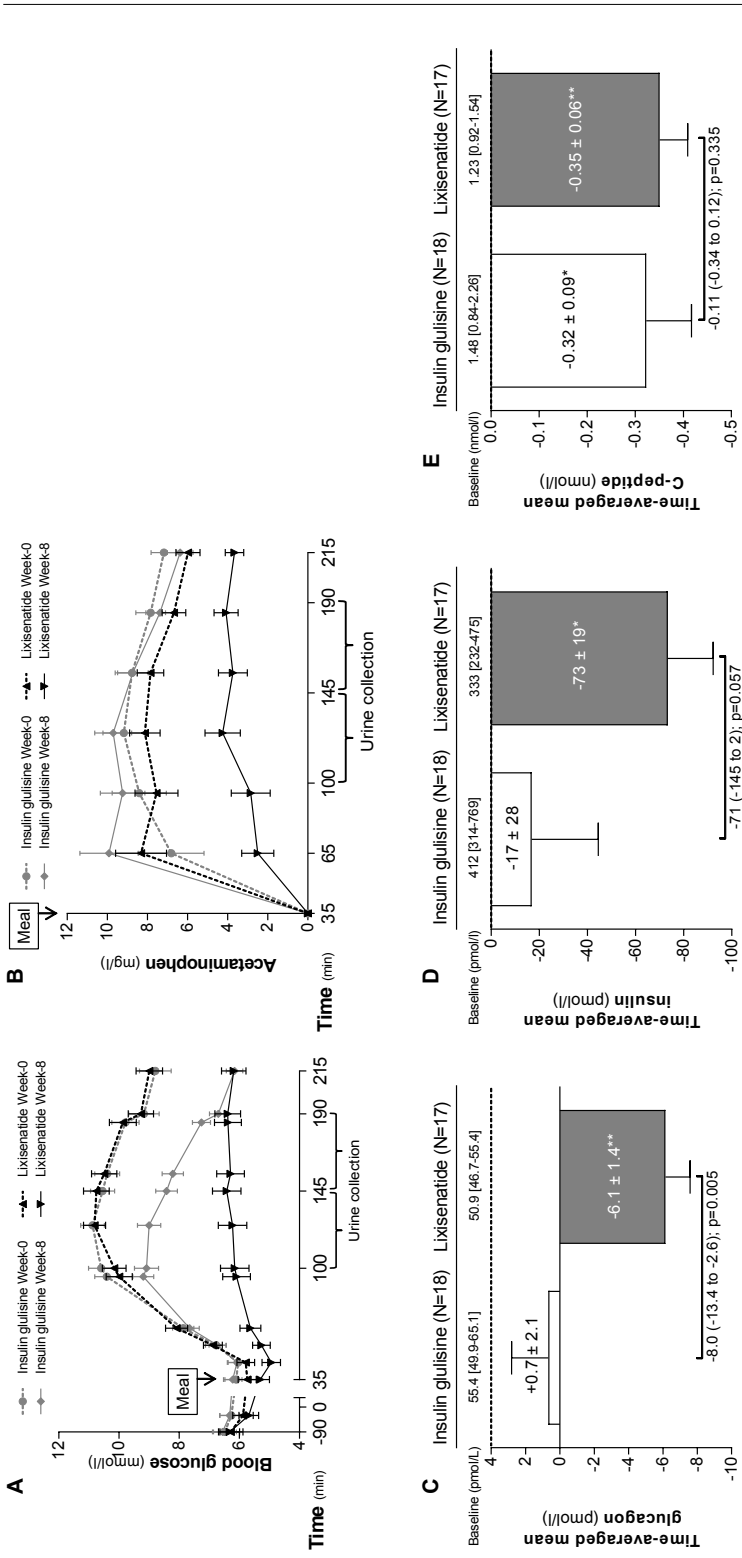


Figure 1. Metabolic and hormonal responses. Data are mean ± SEM (panels A and B) median [IQR] or baseline-corrected mean difference (95% CI) (panels C-E) using multiple linear regression to examine baseline-corrected lixisenatide-induced effects compared to insulin glulisine. Wilcoxon signed rank tests were used for within-group comparisons. *p<0.01. ** p<0.001. Postprandial time-averaged mean values during the renal clearance periods were calculated for glucagon, insulin and C-peptide. Endogenous insulin plus insulin glargine concentrations are determined by insulin assay. The insulin assay does not measure insulin glulisine.

Adverse events

Both treatments were generally well tolerated. In all randomised patients, gastrointestinal adverse events were reported more frequently with lixisenatide [11 patients (55%) vs 0 patients in the iGlu arm, p<0.001; 14 vs 0 total number of events, p=0.003], while the incidence of documented hypoglycaemic events (blood glucose ≤3.9 mmol/L) tended to be higher in patients treated with iGlu [7 (37%) vs 2 (10%) patients, p=0.065; 13 vs 2 total number of events, p=0.134] without cases of severe symptomatic hypoglycaemia. Two serious adverse events occurred:

Table 2. Responses in metabolic measurements, blood biomarkers and body composition

	Insulin glulisine (N=18)		
Variables	**Week-0**	**Week-8**	**Within-group p-value**
Metabolic measurements and blood biomarkers			
HbA_{1c}, %	7.8 ± 0.2	7.2 ± 0.1	**0.001**
HbA_{1c}, mmol/mol	62 ± 2	55 ± 2	**<0.001**
Fasting plasma glucose, mmol/L	7.3 ± 0.5	7.2 ± 0.4	0.923
Time-averaged mean glucose, mmol/L	10.1 [8.7-11.7]	8.0 [7.0-8.9]	**<0.001**
Fasting HDL-C, mmol/L	1.09 ± 0.06	1.11 ± 0.07	0.554
Fasting LDL-C, mmol/L	2.5 ± 0.3	2.4 ± 0.3	0.132
Fasting Total cholesterol, mmol/L	4.4 ± 0.3	4.3 ± 0.3	0.226
Fasting Triglycerides, mmol/L	1.9 [1.0-2.6]	1.5 [1.0-2.4]	0.754
Total cholesterol/HDL-C ratio	4.1 [3.1-4.9]	3.9 [2.9-5.1]	0.255
Triglycerides/HDL-C ratio	1.7 [0.8-2.5]	1.3 [0.7-2.7]	0.879
Amylase, U/L	46.0 [35.5 - 59.0]	43.0 [38.0 - 61.0]	0.842
Lipase, U/L	29.5 [26.5 - 34.0]	30.0 [23.0 - 32.5]	0.622
Albumin, g/L	35.6 ± 0.4	35.1 ± 0.4	0.537
Acetaminophen			
C_{max} minute 0-190, mg/L	9.9 [5.9-14.1]	12.0 [9.2-16.4]	0.231
T_{max} minute 0-190, min	125 [95-155]	95 [65-125]	**0.036**
Auc_{net} minute 0-190, mg/L/3.1hr	1067 [528-1581]	1377 [851-1501]	0.198
Bodyweight and composition			
Bodyweight, kg	95.8 ± 4.2	96.6 ± 4.3	0.119
Waist circumference, cm	115.5 [107.0-122.1]	113.5 [106.0-122.8]	**0.027**
Hip circumference, cm	112.0 [107.8-116.4]	111.8 [104.9-116.8]	0.357
Waist/hip ratio	1.01 ± 0.02	1.02 ± 0.01	0.619
Body fat, %	36.3 [32.4-42.9]	36.4 [32.8-43.4]	0.699
Body water, %	46.5 [41.7-49.1]	46.8 [42.8-49.1]	0.311
$_{\Delta PP\text{-}F}$Body water, %	-0.3 [-0.6 to 0.1]	0.1 [-0.5 to 0.4]	0.278
Blood haematocrit, L/L			
Fasting	0.41 ± 0.01	0.40 ± 0.01	**0.035**
Postprandial minute-145	0.41 ± 0.01	0.40 ± 0.01	0.287

Data are mean ± SEM, median [IQR] or baseline-corrected mean difference (95% CI) using multiple linear regression to examine baseline-corrected lixisenatide-induced effects compared to insulin glulisine. Paired t-tests or Wilcoxon signed rank tests were used for within-group comparisons. Significant differences indicated in bold font. § indicates baseline-corrected ratio (95% CI)

one patient randomised to iGlu experienced two episodes of (new-onset) symptomatic atrial fibrillation after start of the intervention (patient fully recovered after hospital admission). Treatment with lixisenatide did not affect plasma amylase compared with iGlu relative to baseline, while plasma lipase tended to increase by 6.4 U/L (p=0.058; Table 2); no patients had amylase or lipase concentrations >3 times the upper limit of normal. There were no suspected cases of pancreatitis.

Lixisenatide (N=17)				
Week-0	**Week-8**	**Within-group p-value**	**Mean difference Lixisenatide-Insulin glulisine (95% CI) and p-value**	
8.3 ± 0.2	7.5 ± 0.2	**<0.001**	-0.02 (-0.4 to 0.3)	0.897
67 ± 2	59 ± 2	**<0.001**	-0.1 (-4 to 4)	0.961
6.7 ± 0.5	6.4 ± 0.4	0.464	-0.71 (-1.9 to 0.4)	0.213
10.2 [8.9-11.4]	5.7 [5.3-6.7]	**<0.001**	-1.8 (-2.9 to -0.7)	**0.002**
1.13 ± 0.05	1.06 ± 0.05	0.031	-0.08 (-0.16 to 0.01)	0.068
2.0 ± 0.1	2.1 ± 0.2	0.584	0.3 (-0.1 to 0.6)	0.109
3.7 ± 0.2	3.8 ± 0.2	0.408	0.2 (-0.2 to 0.7)	0.269
1.0 [0.8-1.7]	1.1 [0.9-2.0]	0.360	0.1 (-0.2 to 0.5)	0.466
3.2 [3.0-3.7]	3.5 [2.9-4.5]	0.120	0.3 (-0.1 to 0.7)	0.177
0.9 [0.7-1.9]	1.1 [0.7-2.1]	0.163	1.19 [0.90 to1.58)§	0.221
50.0 [38.0 - 64.8]	49.0 [38.5 - 62.5]	0.831	0.4 (-4.1 to 5.0)	0.854
36.00 [28.0 - 44.5]	40.5 [31.0 - 59.8	0.334	6.4 (-0.2 to 13.1)	0.058
36.3 ± 0.5	36.1 ± 0.4	0.579	0.6 (-0.2 to 1.5)	0.155
10.1 [7.0-14.0]	5.5 [2.9-7.1]	**<0.001**	-6.6 (-9.3 to -4.0)	**<0.001**
95 [65-155]	125 [110-185]	**0.017**	41 (12 to 70)	**0.007**
903 [717-1462]	318 [132- 677]	**<0.001**	-748 (-1007 to -489)	**<0.001**
99.2 ± 3.7	98.8 ± 3.7	0.092	-1.4 (-2.5 to -0.2)	**0.025**
108.0 [104.0-117.3]	109.0 [105.3-117.8]	0.421	-1.2 (-2.9 to 0.6)	0.180
111.5 [109.8-114.8]	111.0 [107.5-116.8]	0.306	-1.3 (-3.5 to 0.8)	0.207
0.99 ± 0.02	1.00 ± 0.02	0.349	0.00 (-0.03 to 0.03)	0.971
32.7 [28.6-38.7]	33.3 [29.0-40.7]	0.207	-0.71 (-1.90 to 0.48)	0.232
47.5 [44.1-51.6]	47.4 [44.2-51.2]	0.477	-0.568 (-1.4 to 0.3)	0.187
-0.1 [-0.5 to 0.4]	-0.5 [-0.7 to 0.0)	0.691	-0.6 (-1.3 to 0.02)	0.058
0.41 ± 0.01	0.40 ± 0.01	**0.019**	-0.001 (-0.01 to 0.01)	0.928
0.41 ± 0.01	0.41 ± 0.01	0.538	0.01 (-0.005 to 0.02)	0.232

using multiple linear regression. Postprandial time-averaged mean values during the renal clearance periods were calculated for glucose. Abbreviations: AUC_{net}, net area under the curve; C_{max}, maximal concentration; F, fasting; HDL–C, high–density lipoprotein cholesterol; LDL–C, low–density lipoprotein cholesterol; PP, postprandial; T_{max}, time to reach maximal concentration.

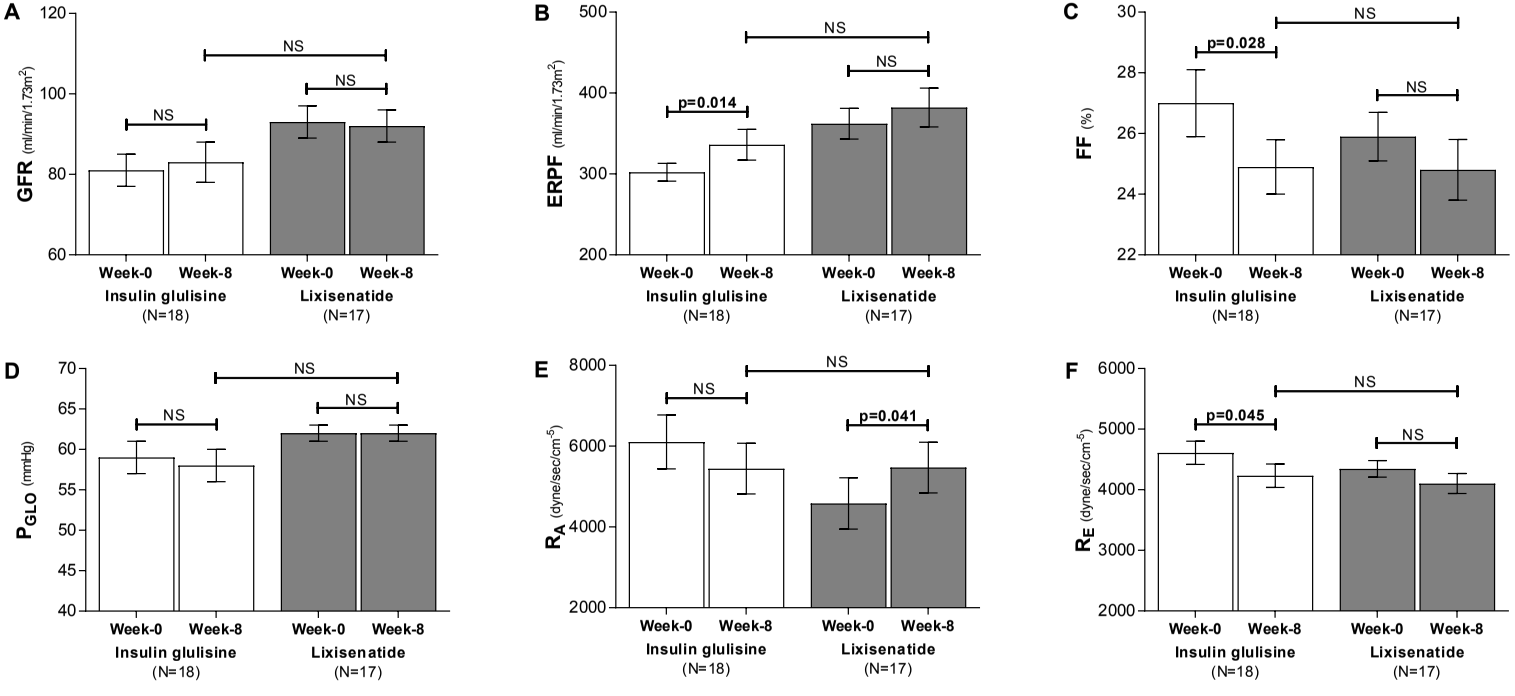
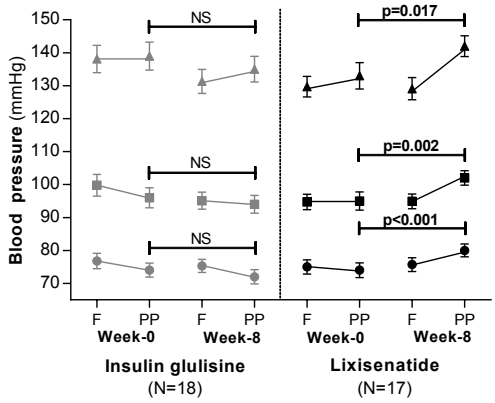


Figure 2. Renal haemodynamic responses. Data are mean ± SEM. Multivariable linear regression models were used to examine baseline-corrected postprandial lixisenatide-induced effects compared to insulin glulisine. Paired t-tests were used for within-group comparisons. Significant differences indicated in bold font. Abbreviations: ERPF, effective renal plasma flow; FF, filtration fraction; GFR, glomerular filtration rate; NS, not significant; P_{GLO}, glomerular hydraulic pressure; R_A, afferent renal arteriolar resistance, R_E, efferent renal arteriolar resistance.



Mean difference Lixisenatide-Insulin glulisine (95% CI)	
▲ **Systolic blood pressure**	
Fasting	3 (-5 to 11), p=0.470
Postprandial	9 (0.6 to 17), **p=0.036**
■ **Mean arterial pressure**	
Fasting	3 (-3 to 8), p=0.331
Postprandial	9 (4 to 14), p=**0.001**
● **Diastolic blood pressure**	
Fasting	2 (-2 to 6), p=0.427
Postprandial	9 (5 to 13), p<**0.001**

Figure 3. Systolic, mean and diastolic blood pressure responses. Data are mean ± SEM or baseline-corrected mean difference (95% CI) using multiple linear regression to examine baseline-corrected lixisenatide-induced effects compared to insulin glulisine in the fasting state (before drug administration) and postprandial state (after drug administration). Paired t-tests were used for within-group comparisons. Significant differences indicated in bold font. Abbreviations: F, Fasting; NS, not significant; PP, postprandial.

Discussion

Eight-week treatment with once-daily prandial lixisenatide compared with once-daily prandial iGlu, added to basal insulin-glargine, did not affect (intra-)renal haemodynamics or renal damage markers after a standardised breakfast in T2DM without overt nephropathy. Lixisenatide led to a sustained natriuretic and urinary alkalising response. Both treatments improved HbA_{1c} to a similar extent, allowing for clinically relevant exploration of glucose-independent properties. Our results are in line with previous head-to-head clinical trials, indicating that improved glycaemic control with lixisenatide compared with once-daily iGlu is reached with a reduction in body weight, fewer hypoglycaemic events and a higher number of gastrointestinal adverse events.[8] Remarkably, lixisenatide compared to iGlu increased blood pressure following meal ingestion.

GLP-1RA have been suggested to reduce hyperfiltration in patients with T2DM by beneficially affecting distinct pathophysiological mechanisms underlying this this phenomenon. First, several small-sized mechanistic studies in humans suggested that acute GLP-1 peptide[10,21] or GLP-1RA[15,16,22] administration inhibits Na+/H+-exchanger isoform-3 (NHE3) activity[23] in the proximal tubule. This theoretically increases sodium chloride delivery to the macula densa and restores the disrupted TGF response associated with diabetes and obesity,[4,24] leading to increased R_A and subsequent reductions in (single-nephron) GFR and ERPF. As such, agents that induce proximal tubular natriuresis, such as sodium-glucose cotransporter inhibitors and carbonic anhydrase inhibitors, have been linked to renoprotective alterations in renal haemodynamics through activation of this autoregulatory mechanism.[4] Second, TGF-mediated afferent vasodilation is further augmented in the postprandial state through increased sodium reabsorption in the proximal tubule (co-transported with glucose and amino acids), and

the thick ascending limb (stimulated by glucagon and vasopressin).[5] In theory, especially short-acting GLP-1RAs (see below) could mitigate this response by means of a sustained suppressive effect on postprandial glucagon concentrations, and a durable delay of gastric emptying rate, which prolongs absorption of meal-derived macronutrients, thereby reducing glucose and amino acid-dependent TGF activation. Third, GLP-1RAs may reduce hyperfiltration by lowering body weight.[1,4] Fourth, as acute GLP-1 peptide and GLP-1RA administration reduces plasma renin activity, PRC and angiotensin-II in some (but not all, including current) trials,[10,15,21,25,26], prolonged GLP-1RA treatment could ameliorate RAAS-mediated hyperfiltration in diabetes.[4] Fifth, we hypothesised that short-acting GLP-1RA could reduce hyperfiltration by reducing

Table 3. Responses in postprandial tubular functions, renal damage markers and RAAS hormones

	Insulin glulisine (*N*=18)		
Variables	**Week-0**	**Week-8**	**Within-group p-value**
Tubular functions			
Plasma sodium			
Fasting	140.0 [138.0-140.3]	139.5 [137.8-143.0]	0.964
Postprandial minute 100-190	139.2 [137.1-140.2]	139.7 137.7 141.8	0.097
Postprandial minute-190	138.9 ± 0.7	139.6 ± 0.7	0.273
Sodium excretion			
Absolute excretion, µmol/min/1.73m^2	108 ± 13	108 ± 8	0.975
Inulin-based FE_{Na}, %	0.96 ± 0.09	0.93 ± 0.07	0.768
$_{\Delta PP\text{-}F}$Creatinine-based FE_{Na}, %	0.03 ± 0.07	-0.11 ± 0.08	**0.047**
Urinary pH	5.24 ± 0.14	5.12 ± 0.09	0.222
$_{\Delta PP\text{-}F}$Urinary pH	-0.3 [-0.8 to -0.1]	-0.2 [-0.8 to 0.1]	0.647
Urinary Flow, mL/min/1.73m^2	3.1 [1.7-4.6]	2.5 [1.6-3.6]	0.071
Renal damage markers			
UACR, mg/mmol	0.93 [0.36-1.68]	0.81 [0.35-2.02]	0.432
$_{\Delta PP\text{-}F}$UACR, mg/mmol	-0.9 [-1.4 to -0.3]	-0.6 [-2.2 to -0.3]	0.532
NGAL/CR, ng/mmol	2790 [1448-6569]	2175 [1059-6222]	0.464
$_{\Delta PP\text{-}F}$NGAL/CR, ng/mmol	131 [-2483 to 764]	33 [-2188 to1642]	0.959
KIM-1/CR, ng/mmol	87 [41-170]	97 [47-140]	0.697
$_{\Delta PP\text{-}F}$KIM-1/CR, ng/mmol	-22 [-61 to 48]	-3 [-47 to 64]	0.448
RAAS hormones			
PRC, pg/ml	11.4 [6.1-13.6]	10.7 [6.9-14.9]	0.874
$_{\Delta PP\text{-}F}$PRC, pg/mL	-0.9 [-1.5 to -0.01]	-0.01 [-1.3 to 0.9]	0.472
Angiotensin-II, fmol/mL	7.7 [5.4-8.7]	8.9 [6.3-10.5]	0.076
$_{\Delta PP\text{-}F}$Angiotensin-II, fmol/mL	-0.2 [-1.3 to 0.6]	0.6 [-1.5 to 1.6]	0.831
Aldosterone, pg/mL	33.9 [24.7-48.5]	27.4 [17.5-51.1]	0.396
$_{\Delta PP\text{-}F}$Aldosterone, pg/mL	-14.6 [-22.8 to -5.9]	-15.9 [-28.3 to -2.9]	0.388

Data are mean ± SEM, median [IQR] or baseline-corrected mean difference (95% CI) using multiple linear regression to examine baseline-corrected postprandial lixisenatide-induced effects compared to insulin glulisine measured during the renal clearance periods or after the renal clearance periods (UACR, NGAL-CR and KIM-1-CR), unless stated otherwise. Paired t-tests or Wilcoxon signed rank tests were used for within-group comparisons. Significant differences indicated in bold font. §indicates

postprandial insulin concentrations (mainly by delaying gastric emptying rate and subsequent intestinal glucose absorption), as insulin *per se* increases renal filtration by reducing R_A.[4,27].

In contrast to our hypothesis, and despite lixisenatide-induced natriuresis (likely of proximal tubular origin), body weight loss, suppression of gastric emptying rate and post-breakfast glucagon and insulin concentrations, treatment with this short-acting GLP-1RA did not result in a clinically relevant effect on postprandial GFR, ERPF, FF or P_{GLO} compared with iGlu. Interestingly, iGlu lowered postprandial FF by increasing ERPF, a pattern most compatible with reduced R_E. Similar albeit non-significant changes were observed with lixisenatide. Additional analyses indicate that these alterations in turn may relate to a decrease in glucose levels and/or

Lixisenatide (*N*=17)				
Week-0	**Week-8**	**Within-group p-value**	**Mean difference Lixisenatide-Insulin glulisine (95% CI) and p-value**	
140.0 [137.5-142.0]	140.0 [138.0-141.5]	0.620	0.3 (-1.0 to 1.5)	0.691
138.3 137.2 139.2	140.3 139.5 142.7	**<0.001**	1.8 (0.4 to 3.2)	**0.011**
138.2 ± 0.6	140.8 ± 0.6	**<0.001**	1.7 (0.1 to 3.3)	**0.034**
105 ± 6	124 ± 10	0.051	18 (-5 to 41)	0.114
0.81 ± 0.04	0.96 ± 0.07	**0.021**	0.11 (-0.07 to 0.28)	0.214
0.08 ± 0.04	0.18 ± 0.05	**0.043**	0.25 (0.09 to 0.41)	**0.003**
5.09 ± 0.09	5.48 ± 0.18	**0.024**	0.45 (0.10 to 0.80)	**0.013**
-0.4 [-0.7 to -0.1]	0.02 [-0.2 to 0.8]	**0.001**	0.7 (0.3 to 1.2)	**0.001**
2.8 [1.4-3.2]	3.0 [2.2-3.6]	0.266	0.7 (-0.1 to 1.4)	0.075
0.45 [0.36-2.19]	0.67 [0.29-1.92]	0.225	1.21 (0.65 to 2.27)§	0.527
-0.4 [-2.3 to 0.01]	-0.2 [-0.9 to -0.02]	0.826	1.00 (0.95 to 1.06)§	0.950
3499 [1326-5339]	1787 [1432-4270]	0.086	0.85 (0.49 to 1.47)§	0.555
211 [-1453 to 1970]	-88 [-1212 to 1982]	0.981	1.00 (0.86 to 1.16)§	0.994
73 [45-130]	64 [35-160]	0.348	-24 (-79 to 30)	0.372
-15 [-64 to 30]	-10 [-30 to 45]	0.890	-16 (-63 to 31)	0.496
7.9 [3.6-27.6]	6.7 [2.9-21.3]	0.149	0.63 (0.40 to 1.00)§	0.052
-0.2 [-1.3 to 0.3]	-1.0 [-5.4 to -0.5]	0.093	1.00 (0.96 to 1.04)§	0.947
7.1 [4.2-9.0]	8.4 [7.0-9.7]	0.102	0.2 (-1.8 to 2.3)	0.815
0.0 [-1.0 to 0.9]	-0.9 [-2.9 to 0.9]	0.234	-1.3 (-3.5 to 1.0)	0.253
29.5 [22.2-47.7]	46.9 [27.2-64.7]	0.193	10.1 (-5.2 to 25.5)	0.189
-18.4 [-37.2 to -8.6]	-4.3 [-18.5 to 14.3]	**0.015**	9.1 (-4.4 to 22.6)	0.178

baseline-corrected ratio using multiple linear regression (95% CI). Abbreviations: CR, creatinine ratio; F, fasting; FE_{Na}, fractional sodium excretion; KIM-1, kidney injury molecule-1; NGAL, neutrophil gelatinase-associated lipocalin; PRC, plasma renin concentration; PP, postprandial; RAAS, renin-angiotensin-aldosterone-system; UACR, albumin/creatinine ratio.

changes in related vasoactive factors that we did not measure.[20] Of note, a potential reduction in R_E through lowering of angiotensin-II may have been blunted by the extensive use of RAS inhibition in the present trial. Additionally, lixisenatide increased R_A, which is in line with a previous study using acute-exenatide infusion in patients with T2DM.[16] However, whether this relates to TGF activation is questionable, as effects on GFR were neutral and R_A did not correlate with FE_{Na}. Moreover, an increase of this estimated variable is very dependent on the increase in MAP in the Gomez equations. After iGlu treatment, R_A tended to decrease, which corresponds with reported effects of insulin *per se*.[20,27]

The previously reported benefits of GLP-1RA on fasting renal haemodynamics in humans with "early phase hyperfiltration" may have been the result of glucose-lowering *per se*, as these were placebo-controlled and did not correct for changes in HbA_{1c} or plasma glucose concentrations. As such, acute GLP-1-peptide infusion reduced creatinine clearance measured GFR from 151 to 142 mL/min in obese males (25% of whom were diagnosed with T2DM), while in parallel also lowering fasting plasma glucose by 1.4 mmol/L.[10] Moreover, 12-week liraglutide treatment in albuminuric T2DM patients improved HbA_{1c} by 0.7%, but did not significantly affect GFR, although GFR decreased >30% in 2 patients with renal function >135 mL/min/1.73 m^2.[13] In contrast, acute GLP-1 peptide-infusion,[14] acute intravenous or single-dose subcutaneous administration of a GLP-1RA[15,16] or 12-week liraglutide treatment[17] did not affect fasting renal haemodynamics in T2DM patients with presumed "late phase hyperfilration" (*i.e.* FF of ~25% in the setting of GFR >60 mL/min/1.73 m^2).[4,15-17]

Lixisenatide had natriuretic and urinary alkalising effects, suggesting sustained GLP-1-receptor-mediated inhibitory effects on NHE3 activity in the proximal tubule. In this respect, it is noteworthy that the natriuretic effects of the long-acting GLP-1RA liraglutide seem to be lost somewhere between 3[15,28] and 12 weeks,[17] which is compatible with receptor desensitisation (or tubular compensatory mechanisms) after prolonged treatment, as is also observed for GLP-1RA in other organ systems.[1,29-31] In contrast, intermittent receptor stimulation with short-acting agents results in preserved efficacy to reduce gastric emptying[30] and postprandial glucagon secretion,[30,32] which presently also seems to hold true for their inhibiting effects on proximal sodium reabsorption. However, a few cautionary comments are worth noting. First, the contrasting effects of liraglutide and lixisenatide could be explained by the fact that only a minor proportion of liraglutide-metabolites passes the glomerular barrier.[17] Second, it may reflect differences in fasting and postprandial natriuretic actions of GLP-1RA. Third, there is uncertainty about the presence of a functional GLP-1 receptor in the human kidney (as commercially available antisera are neither sensitive nor specific, and because of interspecies differences). The study that used the most extensively validated monoclonal antibody for immunohistochemical assessments to date was unable to detect the presence of GLP-1 receptors in monkey and human proximal tubules,[33] calling direct effects of lixisenatide on tubular sodium handling somewhat into question. Fourth, although increases in MAP only partly explained increases in FE_{Na}, we cannot exclude that our finding relates to pressure-natriuresis, which was also acknowledged in a study using acute exenatide-infusion in patients with T2DM.[16] Finally, lixisenatide-mediated effects on distal tubular sodium transporters (with or without

secondary NHE3 involvement) cannot be excluded, which could also explain why the increase in FE_{Na} is not associated with changes in (intra-)renal haemodynamic functions through TGF. Notably, the isolated reduction of FE_{Na} with iGlu is in line with previous studies demonstrating stimulation of distal sodium reabsorption with this hormone.[34]

Long-term treatment with both short- and long-acting GLP-1RA modestly reduces office blood pressure in the fasting state,[35] including a 0.8 mm Hg SBP reduction in the ELIXA measured before lixisenatide administration.[9] In the current study, blood pressure in the fasting state before lixisenatide administration did not change, although postprandial blood pressure increased by 9 mm Hg. This is in line with numerous studies that investigated acute GLP-1[25,36,37] and GLP-1RA administration[15,16,38,39] in the fasting state. Also, following a meal[40] and intraduodenal glucose infusion,[41] blood pressure was higher with acute intravenous exenatide-administration vs placebo in patients with T2DM, although 12-week liraglutide treatment did not affect postprandial haemodynamics.[40] The mechanisms of this response, which only seems sustained after prolonged treatment with a short-acting GLP-1RA, certainly merit further investigation. In the current study, reduced gastric emptying rate likely prevented the hypotensive response normally observed after a meal.[40] In addition, although hypoglycaemia did not occur with lixisenatide and heart rate did not increase, glucose reduction may have led to sympathetic activation[39] and/or vagal suppression,[25] thereby increasing total peripheral resistance.[39] The involvement of RAAS hormones or sympathetic stimulation by insulin is unlikely in the current trial. The increase in blood pressure with lixisenatide could have blunted potential beneficial effects on renal haemodynamics. Notably, the addition of lixisenatide to the standard of care in the ELIXA trial in patients with T2DM with a recent acute coronary syndrome did not alter the rate of major adverse cardiovascular events after 25 months of treatment,[9] indicating that this response does not negatively impact cardiovascular risk.

Our study has several limitations. First, although we aimed to account for glycaemic differences during the renal testing procedures by using an active comparator that specifically reduces postprandial glucose excursions, we did not perform clamp studies. However, both GLP-1RA and pre-prandial insulin preparations are considered treatment options that improve postprandial glycaemic control in patients with T2DM treated with basal insulin.[1] As HbA_{1c} was not different between the treatment groups and hypoglycaemia risk prevented further up-titration of iGlu, the current study design is translational to clinical practice. Second, to minimise the risk of hypoglycaemic events during the testing day in our glargine-treated T2DM population, we did not measure renal haemodynamics in the fasting state, which precludes evaluation of meal-induced changes *per se* on these parameters. Third, the Gomez equations necessitate assumptions, which are insufficiently validated in the postprandial state. Fourth, we did not measure 24-hour sodium excretion or optimally standardise or monitor intake. Although we instructed patients to adhere to a controlled dietary intake of sodium before testing (File S1), dietary alterations could have influenced our results. Yet, as the natriuretic effects of lixisenatide are likely short-lived and restricted to the single meal given its pharmacokinetic profile, compensatory mechanisms to reinstate homeostasis (i.e. match sodium output to intake) may not be activated and tachyphylaxis may not be induced. Future studies should focus

on cumulative urinary sodium excretion after prolonged treatment with long- vs short-acting GLP-1RAs. Fifth, our findings in patients with T2DM with late phase hyperfiltration cannot be generalised to patients with early phase hyperfiltration and/or advanced kidney disease. Sixth, the study had relatively short duration of follow-up and patients had low levels of baseline renal damage, which may explain why neither treatment affected markers of renal damage. Finally, involvement of NHE3 could only be suggested indirectly by the finding of lixisenatide-induced increases in sodium excretion and urinary pH; urinary bicarbonate was not assessed.

In conclusion, 8 weeks treatment with lixisenatide, compared with iGlu, did not affect postprandial (intra-)renal haemodynamics when added to insulin-glargine in patients with T2DM without overt nephropathy. In contrast to long-acting GLP-1RA, prolonged treatment with lixisenatide induced sustained natriuretic and urinary alkalising actions. The (long-term) impact of postprandial increases in blood pressure requires further study. Given the increasing clinical interest in adding a GLP-1RA to basal insulin in comparison to short-acting insulin regimens, the reduction in body weight and lower risk of hypoglycaemia with lixisenatide in our trial may aid clinical decision-making.

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Supplemental methods

Full study protocol

Patients were instructed to adhere to controlled intake of protein (1.5–2.0 g/kg/day) and sodium (9–12 g/day; ~150-200 mmol sodium) 2 days prior to the testing day, to abstain from alcohol ingestion and vigorous physical activity for ≥24 hours, and not to use caffeine or nicotine for ≥12 hours, to reduce variation. After an overnight fast, and before arriving at the clinical research unit of the Diabetes Center of the VU University Medical Center at 07:30 AM, patients consumed 500 mL of tap water to stimulate diuresis. With the exception of metformin and thyroid hormone replacement therapy, all morning medications were delayed. Patients assumed a semi-recumbent position in a temperature controlled-room (23.0 ± 1.0 °C) throughout testing procedures. A venous cannula was inserted in an antecubital vein of the non-dominant arm for venous blood sampling, and in an antecubital vein of the dominant arm for infusion of the renal tracer substances. Before the renal tests, blood samples were taken to determine blood HbA_{1c} and hematocrit, plasma glucose, sodium, creatinine, albumin, lipids (triglycerides, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, amylase, lipase, renin-angiotensin-aldosterone-system (RAAS) hormones (*i.e* plasma renin concentration [PRC], angiotensin-II, and aldosterone), and serum insulin and c-peptide. In addition, a single spot urine specimen was collected to measure sodium, pH, albumin, creatinine, neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1). Then, the renal tests commenced (Figure). Over a 10-minute period, a priming bolus of inulin (Inutest®, Fresenius Kabi Austria GmbH, Graz, Austria; 45 mg/kg bodyweight) and para-aminohippuric acid (PAH) (6 mg/kg bodyweight) was infused. Initially, aminohippurate sodium 'PAH' 20% was obtained from Merck Sharp & Dohme International, Merck & Co. Inc., Whitehouse Station, NJ, but due to discontinuation of product-manufacturing we changed to 4-Aminohippuric Acid Solution 20%, from Bachem Distribution Services GmbH, Weil am Rhein, Germany. After administering the bolus, a continuous infusion was started with inulin at 22.5 mg/min (target plasma concentration 250 mg/l) and PAH at 12.7 mg/min (target plasma concentration 20 mg/l). Starting at minute-35, patients consumed a standardised mixed breakfast within a timeframe of 15 minutes. The meal consisted of bread with jam and cheese, and milk (416 kcal; 14 g fat, 48 g carbohydrates and 22 g protein). Furthermore, together with the meal, 1.5 g of acetaminophen in suspension form (62.5 ml DARO paracetamol, Remark Groep, Rogat, The Netherlands) was administered to assess gastric emptying rate.[1] During the renal testing day at Week-8, patients administered lixisenatide or insulin glulisine (iGlu) (last individually titrated dose plus 20% to account for the maltitol in the acetaminophen suspension) 30 and 10 minutes before the breakfast, respectively. At minute-100, patients emptied their bladder to achieve a zero point for clearance determination, and urine was subsequently collected by spontaneous voiding for two 45–minute periods. Diuresis was prompted by oral intake of 10 ml/kg (maximum 1000 ml) tap water up to minute-90, followed by an intake of 200 ml/h. All patients were seated while voiding, were instructed to use a double voiding technique and reached a subjective feeling of total bladder emptying. Blood samples were taken before and

after each urine collection period to measure inulin, PAH, sodium and creatinine. Additional blood samples were drawn to examine meal-induced changes in blood glucose, serum insulin, C-peptide and acetaminophen. Blood samples for determination of glucagon were collected in BD P800 tubes containing a cocktail of protease, esterase and dipeptidyl peptidase-4 inhibitors (Becton Dickinson, Breda, Netherlands). Hematocrit and RAAS hormones were determined at the midpoint of the two urine collection periods. Intravenous lines were flushed with 2 ml of 0.9% saline after each blood sample, and 0.9% saline was infused at 10 ml/h during the renal tests, corresponding to a total volume load of 54 ml (sodium load ~0.5 g or ~21.7 mmol). Urinary sodium, pH and creatinine were measured during both collection periods and urinary albumin during the second collection period. Urinary NGAL and KIM-1 were additionally measured after the renal tests at minute-235. Body fat percentage was measured before the renal tests and body water percentage before and after the renal tests, directly after bladder emptying, using single-frequency bioelectrical impedance (BF-906, Maltron International Ltd, Essex, UK).

Systolic blood pressure, mean arterial pressure, diastolic blood pressure, and heart rate were determined before the renal tests and drug-administration at minute-120, by an automated oscillometric device (Dinamap©, GE Healthcare, Little Chalfont, UK) at the brachial artery of the non-dominant arm. Measurements were performed in triplicate at 1-2 min intervals, using the mean of the last two measurements.

After randomisation, patients were instructed to consume a daily breakfast with a comparable composition as during the test-procedures.

Assays

Inulin and PAH were analysed as described.[2] Blood HbA_{1c} and hematocrit, plasma glucose, sodium, creatinine, albumin, lipids, amylase and lipase were assayed at the department of clinical chemistry in our hospital by conventional methods. Blood glucose was determined using a YSI-2300 STAT Glucose analyser (YSI Life Sciences, Yellow Springs, OH). Insulin and C-peptide were measured using immunometric assays (Advia Centaur XP, Siemens Medical Solutions Diagnostics, Malvern, PA). The insulin assay has low cross-reactivity with iGlu (1%, 2% and 15% at iGlu concentrations of 829 pmol/l, 166 pmol/l and 32 pmol/l, respectively) and high cross-reactivity with insulin glargine (150%, 136%, 147%, 142% and 134% at insulin glargine

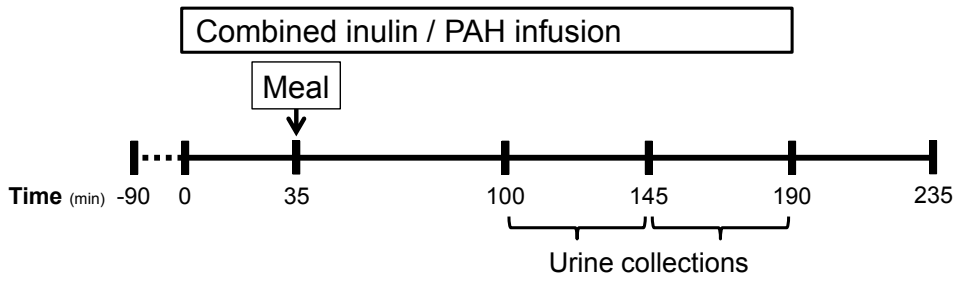

Figure. Study design

concentration of 1600 pmol/l, 640 pmol/l, 290 pmol/l, 78 pmol/l and 32 pmol/l, respectively). Plasma glucagon was measured by radioimmunoassay (Euro Diagnostica AB, Malmö, Sweden). Acetaminophen was determined using an enzymatic immunoassay (Seksisui Diagnostics, Allington, Maidstone, UK) on an Architect 4000 analyser (Abbott Laboratories, Abbott Park, IL). PRC was measured with an immunoradiometric kit (Renin III; Cisbio, Gif-sur-Yvette, France). Angiotensin-II was determined by radioimmunoassay after SepPak extraction. Aldosterone was measured by solid-phase radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA). Urinary pH was determined by hand-held VARIO® 2V00 pH-meter and SenTix-V electrode (Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany). Urinary NGAL and KIM-1 were measured by sandwich ELISA (R&D Systems, Minneapolis, MN).

Calculation of renal physiology and markers of renal damage

Calculations for glomerular filtration rate (GFR), effective renal plasma flow, filtration fraction, renal blood flow, renal vascular resistance, intrarenal haemodynamics (i.e. glomerular hydraulic pressure and afferent and efferent renal arteriolar resistance; for this, given the mean GFR of 86.7 mL/min/1.73 m^2 in the current population, we assumed a gross filtration coefficient of 0.0577 ml/sec/mmHg) and absolute sodium excretion have been described.[2] Fractional sodium excretion (FE_{Na}) during renal tests was calculated using inulin as reference substance, while creatinine was used to calculate FE_{Na}-difference during the postprandial and fasting state. Renal haemodynamic variables were corrected for body surface area, using the Mosteller formula.[3] For estimated GFR we used the chronic kidney disease epidemiology collaboration 2009 and modification of diet in renal disease equations (www.kidney.org/professionals/kdoqi/gfr_calculator). Urinary albumin, NGAL and KIM-1 were corrected for urinary creatinine. Albuminuria/creatinine ratio (UACR) measured before the renal tests was categorized to normo-, micro- and macroalbuminuria according to KDIGO standards[4] and regression or progression between categories was noted, while percentage change in UACR was calculated. Postprandial time-averaged mean values during the renal clearance periods were calculated for glucose, insulin, C-peptide and glucagon. For acetaminophen, the net area under the curve, maximal concentration (C_{max}) and time to reach C_{max} were calculated.

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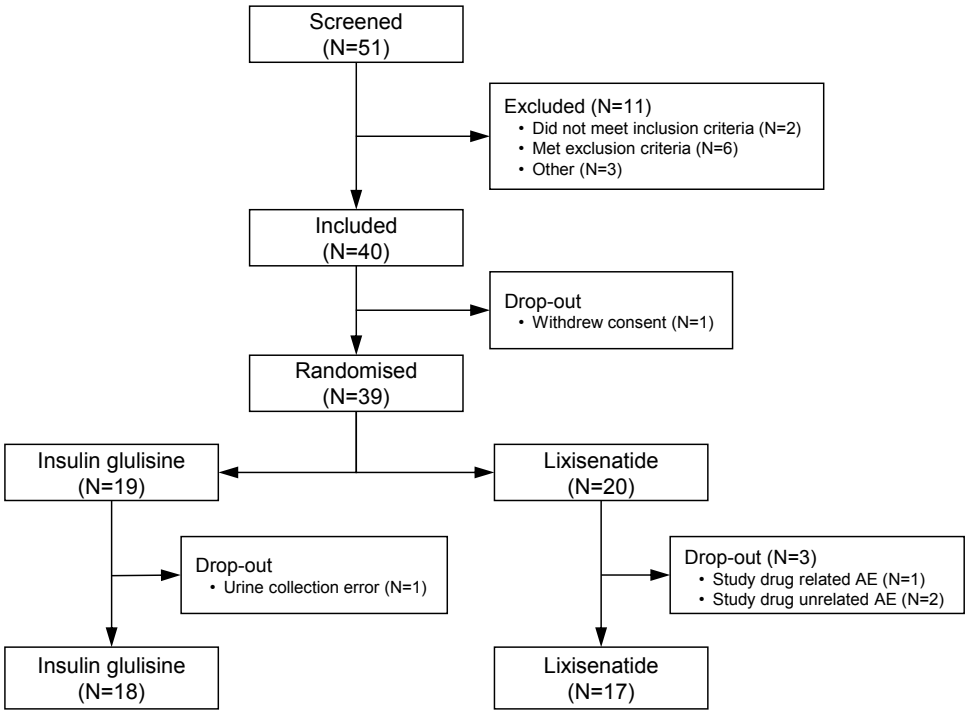
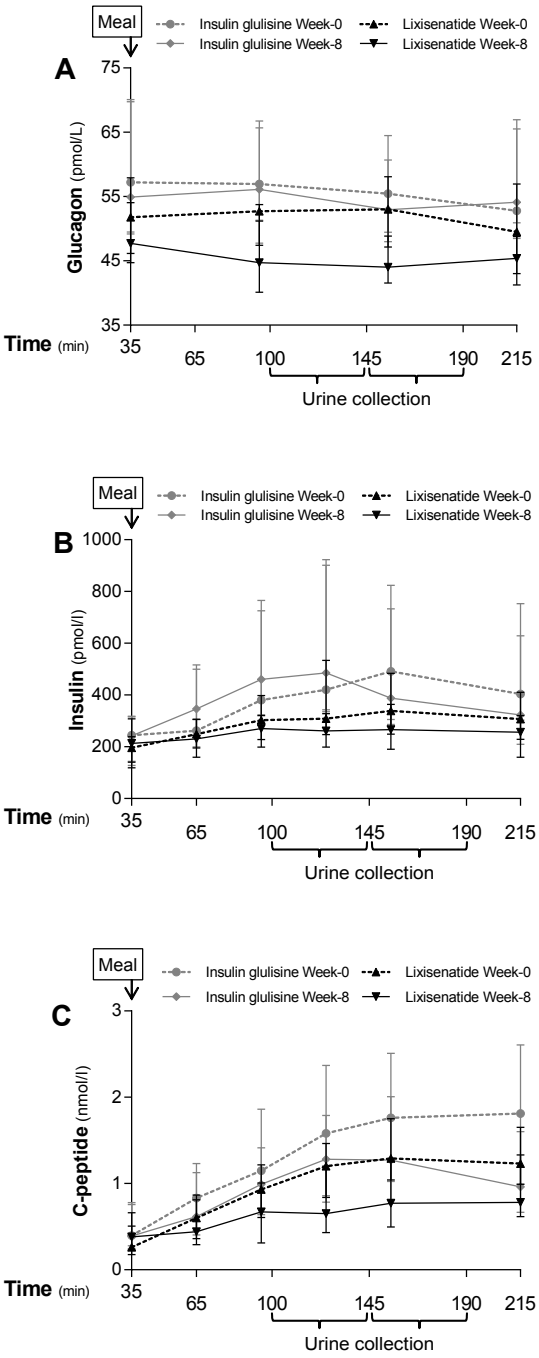
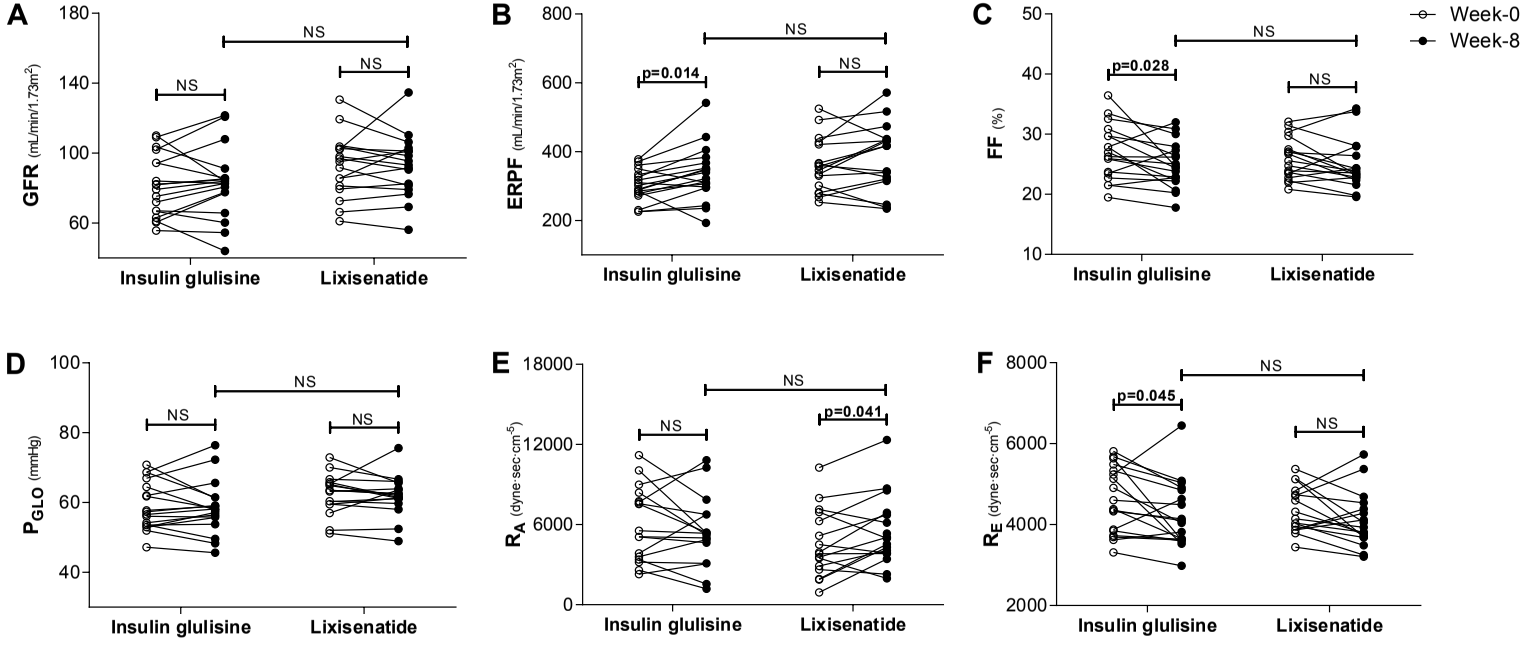
Supplemental data



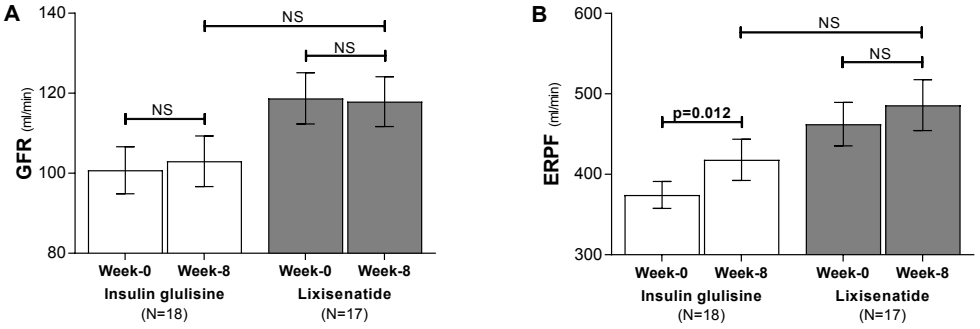
Supplemental Figure 1. Flow diagram of participants in the study



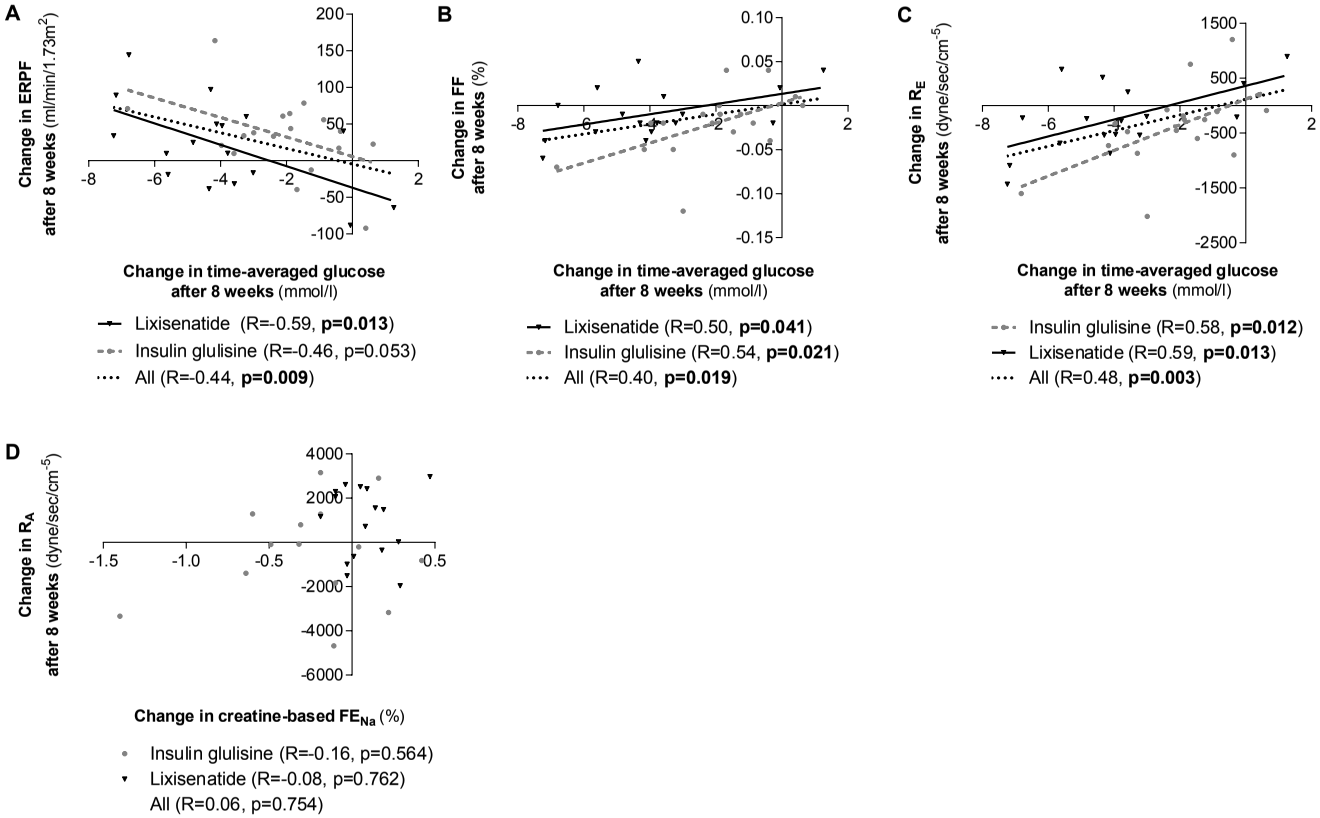
Supplemental Figure 2. Hormonal responses. Data are median [IQR]. Postprandial time-averaged mean values during the renal clearance periods were calculated for glucagon, insulin and C-peptide. Endogenous insulin plus insulin glargine concentrations are determined by the insulin assay. The insulin assay does not measure insulin glulisine.



Supplemental Figure 3. Individual renal haemodynamics responses. Data are absolute values. Multivariable linear regression models were used to examine baseline-corrected postprandial lixisenatide-induced effects compared to insulin glulisine. Paired t-tests were used for within-group comparisons. Significant differences indicated in bold font. Abbreviations: ERPF, effective renal plasma flow; FF, filtration fraction; GFR, glomerular filtration rate; NS, not significant; P_{GLO}, glomerular hydraulic pressure; R_A, afferent renal arteriolar resistance, R_E, efferent renal arteriolar resistance.



Supplemental Figure 4. Body surface *uncorrected* renal haemodynamics responses. Data are mean ± SEM. Multivariable linear regression models were used to examine baseline-corrected postprandial lixisenatide-induced effects compared to insulin glulisine. Paired t-tests were used for within-group comparisons. Significant differences indicated in bold font. Abbreviations: ERPF, effective renal plasma flow; GFR, glomerular filtration rate.



Supplemental Figure 5. Correlations of within-group changes in (intra-)renal haemodynamics. Correlations between within-group changes in postprandial (intra-)renal haemodynamic and postprandial time-averaged glucose during the renal clearance periods, in addition to changes in creatinine-based FE_{Na} (difference during the postprandial and fasting state) using Pearson's correlation (A-C) or Spearman signed rank test (D). Significant correlations indicated in bold font. Abbreviations: ERPF, effective renal plasma flow; FE_{Na}, fractional sodium excretion; FF, filtration fraction; R_A, afferent renal arteriolar resistance; R_E, efferent renal arteriolar resistance.

Supplemental Table 1. (Intra-)renal haemodynamic responses

	Insulin glulisine (N=18)		
Variables	**Week-0**	**Week-8**	**Within-group p-value**
Measured renal haemodynamics			
GFR, mL/min/1.73m^2	81 ± 4	83 ± 5	0.589
ERPF, mL/min/1.73m^2	302 ± 11	336 ± 19	**0.014**
RBF, mL/min/1.73m^2	510 ± 21	564 ± 33	**0.016**
FF, %	27.0 ± 1.1	24.9 ± 0.9	**0.028**
RVR, mmHg/l/min	0.200 [0.159-0.213]	0.173 [0.142-0.210]	**0.044**
Estimated intra-renal haemodynamic			
$P_{GLO,}$ mmHg	59 ± 2	58 ± 2	0.782
R_A, dyne/sec/cm^{-5}	6104 ± 668	5443 ± 628	0.331
R_E, dyne/sec/cm^{-5}	4609 ± 192	4232 ± 194	**0.045**
Estimated fasting GFR			
CKD-EPI 2009, mL/min/1.73m^2	82 ± 3	82 ± 2	0.839
MDRD, mL/min/1.73m^2	78 ± 3	77 ± 2	0.625

Data are mean ± SEM, median [IQR] or baseline-corrected mean difference (95% CI) using multiple linear regression to examine baseline-corrected postprandial (unless stated otherwise) lixisenatide-induced effects compared to insulin glulisine. Paired t-tests or Wilcoxon signed rank tests were used for within-group comparisons. Significant differences indicated in bold font. Patients randomised to lixisenatide tended to have a higher baseline postprandial GFR (p=0.064) and fasting eGFR-MDRD (p=0.052), whereas postprandial ERPF and RBF were significantly higher in the lixisenatide-arm at baseline (P=0.009

Lixisenatide (N=17)				
Week-0	Week-8	Within-group p-value	Mean difference Lixisenatide-Insulin glulisine (95% CI) and p-value	
93 ± 4	92 ± 4	0.912	0.1 (-9 to 9)	0.989
362 ± 19	382 ± 24	0.179	-17 (-61 to 26)	0.423
612 ± 30	652 ± 41	0.167	-24 (-102 to 53)	0.528
25.9 ± 0.8	24.8 ± 1.0	0.136	0.6 (-1.5 to 2.8)	0.556
0.157 [0.130-0.195]	0.154 [0.134-0.192]	0.352	0.020 (-0.005 to 0.046)	0.116
62 ± 1	62 ± 1	0.623	0.4 (-3 to 4)	0.774
4581 ± 635	5470 ± 629	**0.041**	1.081 (-282 to 2444)	0.116
4345 ± 137	4103 ± 166	0.132	30 (-419 to 478)	0.893
89 ± 3	89 ± 2	0.932	2 (-2 to 6)	0.343
87 ± 3	86 ± 3	0.773	3 (-2 to 8)	0.224

and P=0.008, respectively); no between-group difference in other baseline (intra-)renal haemodynamic characteristics were seen before treatment. Abbreviations: CKD-EPI, chronic kidney disease epidemiology collaboration; ERPF, effective renal plasma flow; FF, filtration fraction; GFR, glomerular filtration rate; MDRD, modification of diet in renal disease; P_{GLO}, glomerular hydraulic pressure; R_A, afferent renal arteriolar resistance; R_E, efferent renal arteriolar resistance; RVR, renal vascular resistance.

Supplemental Table 2. Blood pressure and heart rate responses

	Insulin glulisine (N=18)		
Variables	**Week-0**	**Week-8**	**Within-group p-value**
Systolic blood pressure, mmHg			
Fasting	138 ± 4	131 ± 4	**0.028**
Postprandial	139 ± 4	135 ± 4	0.339
Postprandial-Fasting	0 ± 2	4 ± 2	0.301
Mean arterial pressure, mmHg			
Fasting	100 ± 3	95 ± 3	0.054
Postprandial	96 ± 3	94 ± 3	0.277
Postprandial-Fasting	-4 ± 1	-2 ± 1	0.266
Diastolic blood pressure, mmHg			
Fasting	77 ± 2	75 ± 2	0.319
Postprandial	74 ± 2	72 ± 2	0.173
Postprandial-Fasting	-3 ± 1	-4 ± 1	0.495
Heart rate, beats/min			
Fasting	68 ± 2	66 ± 2	0.265
Postprandial	71 ± 3	70 ± 2	0.379
Postprandial-Fasting	3 [1 to 6]	4 [2 to 7]	0.490

Data are mean ± SEM, median [IQR] or baseline-corrected mean difference (95% CI) using multiple linear regression to examine baseline-corrected lixisenatide-induced effects compared to insulin glulisine in the fasting state before drug administration

Lixisenatide (N=17)				
Week-0	**Week-8**	**Within-group p-value**	Mean difference Lixisenatide-Insulin glulisine (95% CI) and p-value	
130 ± 3	129 ± 3	0.845	3 (-5 to 11)	0.470
133 ± 4	142 ± 3	**0.017**	9 (0.6 to 17)	**0.036**
4 ± 2	12 ± 2	**0.008**	8 (1 to 14)	**0.018**
95 ± 2	95 ± 2	0.907	3 (-3 to 8)	**0.331**
95 ± 3	102 ± 2	**0.002**	9 (4 to 14)	**0.001**
0 ± 2	8 ± 2	**0.012**	8 (3 to 14)	**0.004**
75 ± 2	76 ± 2	0.673	2 (-2 to 6)	0.427
74 ± 2	80 ± 2	**<0.001**	9 (5 to 13)	**<0.001**
-2 ± 1	5 ± 2	**0.007**	8 (4 to 12)	**0.001**
65 ± 2	66 ± 2	0.854	0.2 (-3 to 3)	0.912
68 ± 2	68 ± 2	0.940	0.2 (-3 to 3)	0.912
2 [-1 to 5]	3 [-2 to 7]	0.735	-2 (-6 to 1)	0.160

and postprandial state after drug administration state. Paired t-tests or Wilcoxon signed rank tests were used for within-group comparisons. Significant differences indicated in bold font.