Appendix S1│Materials and Methods

Trial Design and Population

The RENALIS (RENoprotection in diAbetes by LInagliptin versus Sulfonylurea) trial was a phase-IV, randomized, double-blind, comparator-controlled, parallel-group, mechanistic intervention trial, conducted between May-2014 and April-2016 at the Amsterdam University Medical Centers, location VUMC, Amsterdam, The Netherlands. The trial-protocol and its amendments were approved by the local institutional review board and ethics committee, competent local authorities, complied with the Declaration of Helsinki and Harmonized Tripartite Guideline for Good Clinical Practice, and registered with ClinicalTrials.gov (NCT02106104). Written informed consent was obtained from all patients.

Patients were recruited by advertisements in local newspapers. The complete list of eligibility-criteria is provided in BOX 1 In short, eligible patients were Caucasian, men or post-menopausal women, aged 35-75 years, had T2DM, were receiving metformin alone or low-dose sulfonylurea (that could be safely washed-out), had an HbA1c 6.5-9.0% (48-75 mmol/mol) on metformin alone and a BMI ≥25 kg/m^2. In case of hypertension (defined as >140/90 mmHg) and/or albuminuria, treatment included a RAS-blocker (stable dose) for ≥3 months. The main exclusion criteria were history of pancreatic, active liver or malignant disease, estimated glomerular filtration rate ([e]GFR) <60mL/min/1.73m^2, urinary retention (complete bladder emptying was objectified by bladder-ultrasonography at screening), or use of diuretics that could not be stopped 3 months prior to and during the intervention.

BOX 1│Inclusion and Exclusion Criteria

Inclusion criteria

- Caucasian
- Male and Female (must be post-menopausal, defined as no menses >1 year; in case of doubt, follicle-stimulating hormone will be determined with cut-off defined as >31 U/L)
- Age: 35 - 75 years
- BMI: >25 kg/m^2
- HbA1c: 6.5 – 9.0 % DCCT or 48 - 75 mmol/mol IFCC
- Treatment with a stable dose of oral glucose-lowering agents for at least 3 months prior to inclusion
- Metformin monotherapy
- Combination of metformin and low dose SU derivative*
- All patients with previously diagnosed hypertension should use a RAS-interfering agent for at least 3 months**

* In order to accelerate inclusion, patients using combined metformin/SU derivative will be considered. In these patients, a 12 week wash-out period of the SU derivative will be observed, only when combined use has led to a HbA1c <8% at screening. Subsequently, patients will be eligible to enter the study, now using metformin monotherapy, provided that HbA1c still meets inclusion criteria.

** Patients not previously diagnosed but -at screening- fulfilling the criteria of hypertension (i.e. BP >140/90 mmHg, after careful evaluation) will first be treated with a RAS-interfering agent, at a stable dose for a period of 12 weeks, during which BP <140/90 mmHg should be achieved in order to render them eligible for the study

Exclusion criteria

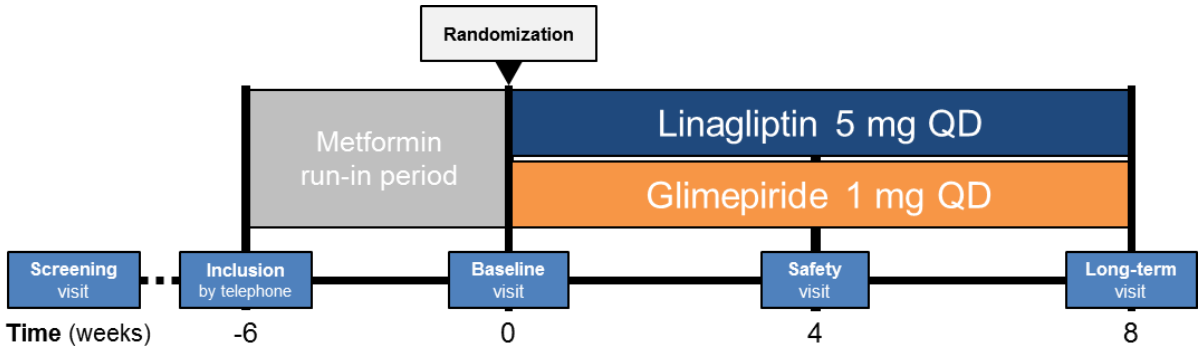
- Current / chronic use of the following medication: thiazolidinediones, insulin, glucocorticoids, immune suppressants, antimicrobial agents or chemotherapeutics. Subjects on diuretics will only be excluded when these drugs (e.g. hydrochlorothiazide) cannot be stopped for the duration of the study

- Chronic use of NSAIDS will not be allowed, unless used as incidental medication (1-2 tablets) for non-chronic indications (i.e. sports injury, head-ache or back ache). However, no such drugs can be taken within a time-frame of 2 weeks prior to renal-testing
- Estimated Glomerular Filtration Rate < 60 mL/min/1.73m2 (determined by the Modification of Diet in Renal Disease (MDRD) study equation)
- Pregnancy
- Frequent occurrence of (confirmed) hypoglycemia (plasma glucose 3.9 mmol/L)
- Current urinary tract infection and active nephritis
- Recent (<6 months) history of cardiovascular disease, including:
- Acute coronary syndrome
- Chronic heart failure (New York Heart Association grade II-IV)
- Stroke
- Transient ischemic neurologic disorder
- Complaints compatible with or established gastroparesis
- Active liver disease or a 3-fold elevation of liver enzymes (aspartate aminotransferase (AST) / alanine aminotransferase (ALT)) at screening
- History of or actual pancreatic disease
- History of or actual malignancy (except for basal cell carcinoma)
- History of or actual severe mental disease
- Substance abuse (alcohol: defined as >4 units/day)
- Allergy to any of the agents used in the study
- Individuals who are investigator site personnel, directly affiliated with the study, or are immediate (spouse, parent, child, or sibling, whether biological or legally adopted) family of investigator site personnel directly affiliated with the study
- Inability to understand the study protocol or give informed consent

Intervention and Randomization

Before randomization, patients using metformin monotherapy were enrolled in a 6-week run-in period (Figure S1A); those receiving metformin and a low-dose sulfonylurea entered a 6-week washout period followed by the 6-week run-in. Patients were then randomly assigned in a 1:1 ratio (block-size 4; performed by an independent trial-pharmacist using computer-generated numbers), in a double-blind fashion, to receive linagliptin 5mg QD (Boehringer Ingelheim, Ingelheim am Rhein, Germany) or glimepiride 1mg QD added to ongoing metformin (dose unchanged throughout the study). Patients were instructed to take their study-drug daily at the same time in the evening. The study-drugs were over-encapsulated, producing visually identical oral capsules by an independent GMP-certified clinical research organization (ACE-Pharmaceutical, Zeewolde, The Netherlands); patients and investigators remained blinded to treatment-status until database-unlock.

Figure S1A | Trial design and treatment



Study Endpoints

The predefined co-primary endpoint was linagliptin-induced changes in GFR and effective renal plasma flow (ERPF) from baseline to Week-8, compared to glimepiride, as derived from inulin and PAH-clearances. All other (intra)renal variables, tubular functions and blood pressure (BP) were considered secondary endpoints. Changes in bodyweight, hematocrit, body water percentage, HbA_{1c}, blood glucose, lipid profiles, renin concentration, insulin, glucagon, DPP-4-activity, DPP-4-substrates (i.e. total and intact GLP-1, substance-P, active and pro neuropeptide-Y [NPY], and stromal cell-derived factor-1α [SDF-1 α]), and hypoglycemia were analyzed as safety and/or exploratory endpoints.

Study Protocol

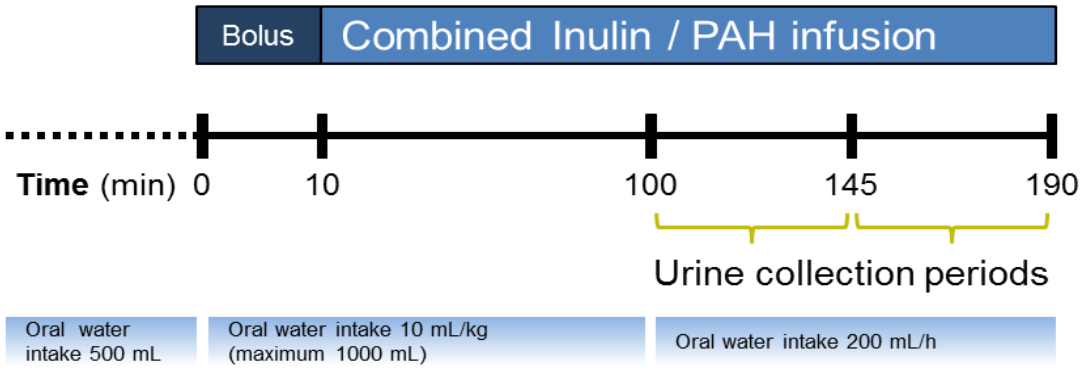
Two days prior to the study visits, patients were instructed to adhere to a controlled sodium chloride (9–12 mg/day; ~150–200 mmol sodium/day) and protein (1.5–2.0 mg/kg/day) diet, to reduce variation in renal physiology. In addition, prior to the experiments, participants were instructed to refrain from vigorous physical activity and alcohol consumption for ≥24 hours, and not to use nicotine or caffeine-containing products for ≥12 hours. After an overnight fast, patients drank 500mL of tap water (to stimulate diuresis) before arriving at the clinical research unit (CRU) at 07:30AM. 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 the testing-day. Intravenous catheters were inserted in an antecubital vein of both forearms to allow intermittent blood sampling on the one side, and continuous infusion of inulin and PAH on the other. Before the renal tests, blood samples were taken to determine creatinine and plasma glucose, HbA1c, lipids and PRC. In addition, a single spot urine specimen was collected to measure creatinine, urinary-pH, albumin, NGAL and KIM-1.

Subsequently, the renal tests commenced (see Figure S1B). GFR and ERPF were determined by standard-method renal clearance technique based on timed urine-sampling using inulin (Inutest®, Fresenius-Kabi Austria GmbH, Graz, Austria) and PAH (initially Aminohippurate sodium 'PAH' 20% by Merck Sharp & Dohme International, Whitehouse Station, NJ; but due to discontinuation of product-manufacturing we switched to 4-Aminohippuric Acid Solution-20%, Bachem Distribution Services GmbH, Weil am Rhein, Germany), respectively. After an acclimatization period of ~50 minutes, a 10-minute priming-infusion of inulin (45 mg/kg bodyweight) and PAH (6 mg/kg bodyweight) was administered, immediately followed by a constant infusion rate 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). After a 90-minute of equilibration, 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 1000mL) tap water during the 90-minute inulin/PAH-equilibration period, followed by an intake of 200 mL/h. All patients were seated while voiding, were instructed to use a double-voiding technique and were encouraged to reach a subjective feeling of total bladder-emptying. Urine volume was recorded to the nearest 1mL. Aliquots were drawn from each collection and analyzed with respect to inulin, PAH, electrolytes, urea, osmolality and pH. Venous blood samples were drawn before and after each urine collection period for assay of inulin, PAH, electrolytes and urea. Hematocrit was determined at the midpoint of the two urine collection-periods. Blood was taken for PRA after 30 minutes of rest. Details on the assays used are described in Appendix-S1D. Intravenous lines were flushed with 2 mL of 0.9% saline after each blood sampling, and a 0.9% saline infused-rate of 10 mL/h was sustained during the renal tests, corresponding to a total volume load of 38 mL and a sodium load of ~0.3 g.

Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and heart rate was measured at on arrival at the CRU and during the renal tests, by an automated oscillometric device (Dinamap©, GE Healthcare, Little Chalfont, UK) over the brachial artery of the non-dominant arm. Measurements were performed in triplicate at 1-2 min intervals and the mean of the last two measurements was used for analyses. Body water percentage was assessed before and during renal testing, using single-frequency bioelectrical impedance (BF-906, Maltron International Ltd, Essex, UK). Probable symptomatic hypoglycemia was defined as an event during which symptoms of hypoglycemia were not accompanied by a plasma glucose determination, but that was presumably caused by a plasma glucose concentration ≤3.9mmol/L.

Figure S1B | Study Protocol



Assays

Venous blood was drawn from the intravenous cannula using syringes, directly transferred to designated BD Vacutainer® tubes (Franklin Lakes, NJ) and centrifuged. For measurements of serum insulin, blood was collected into tubes containing serum clot activator and was left to coagulate for at least 20 min at room temperature. For analyses of glucagon and DPP-4 substrates, blood were

collected into chilled BD™ P800 Blood Collection and Preservation System tubes containing a proprietary cocktail of protease, esterase and dipeptidyl peptidase IV inhibitors that immediately solubilizes during blood collection (Becton Dickinson, Breda, Netherlands), and placed on ice immediately after sampling. Serum and plasma samples were stored at −20°C or −80°C, until batch analyses could be performed.

Fasting plasma glucose, HbA_{1c} (high-performance liquid chromatography) and other baseline laboratory tests were measured before the renal experiments. Venous blood glucose was measured using a YSI-2300 STAT Glucose analyzer (YSI Life Sciences, Yellow Springs, OH, USA) throughout the study, fasting plasma glucose was measured using the Gluco-Quant-hexokinase method on a Modular-P (Roche Diagnostics, Basel, Switzerland). Sodium, potassium, urea, creatinine, albumin, HbA_{1c} and lipids were assayed at the Department of Clinical Chemistry at the Amsterdam University Medical Centers, location VUMC, by conventional methods. Hematocrit was determined using the automated Cell-Dyn Sapphire (Abbott Diagnostics, Abbott Park, IL). Urinary pH was determined by hand-held VARIO® 2V00 pH-meter and SenTix-V electrode (WissenschaftlichTechnischeWerkstätten GmbH, Weilheim, Germany). Urinary albumin levels were measured using immunonephelometric techniques. Heparine-plasma and urine, stored at -80^{0}C before assay, were used to assess inulin and PAH by colorimetric assay after preparation with p-dimethylamino-benzaldehyde for inulin or trichloroacetic acid and indole-3-acetic acid for PAH. Urine concentrations of KIM-1 and NGAL were determined by sandwich ELISA according to manufacturer's specification (R&D Systems, Minneapolis, MN). The intra- and inter-assay variations of NGAL are 4.1% and 3.1%, respectively and for KIM-1, the variations are 8.8% and 10.7%, respectively. Plasma renin concentration was measured with a commercial immunoradiometric kit (Renin III; Cisbio, Gif-sur-Yvette, France). Plasma lipase (pancreas-specific; normal value <70 U/L) and amylase (α-amylase, measuring pancreatic and salivary amylase; normal value <100 U/L) were measured using standardized enzymatic techniques, according to the International Federation of Clinial Chermistry. Plasma insulin was measured using immunometric assays (Advia Centaur XP, Siemens Medical Solutions Diagnostics, Malvern, PA). Plasma glucagon was measured by radioimmunoassay (Euro Diagnostica AB, Malmö, Sweden). SDF-1α in platelet-poor plasma were measured using a sensitive (47 pg/mL) Solid Phase Sandwich ELISA Kit (Human CXCL12/SDF-1 alpha Quantikine ELISA Kit, R&D System, Inc. Minneapolis, MN) according to the manufacturer's instructions; mean CV% are 3.6 (for intra-assay precision) and 10.3 (for inter-assay precision). Plasma pro-NPY and active-NPY were measured using a Human Neuropeptide Y EIA Kit (RayBiotech, Inc., Norcross, GA) according to the manufacturer's instructions. Active substance-P in plasma was measured using a sensitive competitive ELISA Kit (Parameter™ Substance P Assay Kit, KGE007; R&D Systems, Inc. Minneapolis, MN) according to the manufacturer's protocol; mean CV% are 6.25 (for intra-assay precision) 11.9 (for inter-assay precision). DPP-4 activity was measured using the DPPIV-Glo™ protease assay (Promega, Germany) according to the manufacturer's instructions. Before GLP-1 measurement, all samples were extracted in a final concentration of 70% ethanol. Total GLP-1 was measured as described in by Ørskov et al (Diabetes 1994, 43:535-39) using a radioimmunoassay specific for the C-terminal of the GLP-1 molecule (antibody code no 89390) and reacting equally with intact GLP-1 and the primary (N-

terminally truncated) metabolite. Biologically active intact GLP-1 was measured using an in-house (Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark) sandwich ELISA specific for the 7-36NH2 form as previously described and validated (Wewer Albrechtsen et al. Endocr Connect 2015;4(1):50-7). Urinary excretion of very low levels of endogenous lithium was measured by inductively coupled plasma-mass spectrometry (ICP-MS); as this was an exploratory endpoint, we assessed FE_{Li} only in those patients that were treated with linagliptin. Since no Lithium-free urine exist, samples were analysed using standard addition with low amounts of Lithium. A calibration line ranging from 5–50 microg/L was created with a LLoQ of 5 microg/L and a CV <15% over the entire working range (EMA requirements).

Calculation of Renal Physiology Endpoints and Markers of Kidney Damage

GFR and ERPF were calculated from inulin and PAH-clearances, respectively, based on timed urine sampling and averaged from consecutive urine collection-periods. Renal blood flow (RBF) was calculated as ERPF/(1−hematocrit), filtration fraction (FF) as GFR/ERPF, and renal vascular resistance as MAP/RBF. Intrarenal hemodynamics (i.e., P_{GLO} and afferent and efferent arteriolar resistance [R_A and R_E, respectively]) were estimated according to the Gomez formulae (see below). Fractional sodium (FE_{Na}), endogenous lithium ($FE_{Lithium}$; only assessed in linagliptin-treated patients), potassium (FE_K), and urea (FE_U) excretion were calculated using inulin as reference substance. Urinary albumin, neutrophil gelatinase-associated lipocalin (NGAL), and kidney injury molecule (KIM)-1 were corrected for creatinine. Renal hemodynamic variables were corrected for body surface area using the Mosteller formula.(9)

Calculation of intra-renal hemodynamics

Intrarenal hemodynamics were estimated according to the model originally described by Gomez et al. Filtration pressure across the glomerular capillaries (ΔP_F) is calculated by the following Gomez-formula, with the gross filtration coefficient (K_{FG}) assumed to be 0.0554 mL/sec/mmHg (given a normal kidney physiology where GFR is 83.4 mL/min, i.e. mean of the current population), P_{GLO} is 60 mmHg (given Winton's indirect estimates in the dog that glomerular pressure is roughly two-thirds of MAP), and normal glomerular oncotic pressure (πG) is 25 mmHg:

$$\Delta P_F = GFR\ (mL/sec)/\ K_{FG}$$

πG (mmHg) is obtained from CM (plasma protein concentration within the glomerular capillaries), and calculated from TP (total protein concentration; g/dL) and FF:

$$CM = TP/FF * Ln\ (1/1 - FF)$$

$$\pi G = 5 * (CM - 2)$$

P_{GLO} was calculated by using above calculated variables and given the assumption that hydrostatic pressure in Bowman's space (P_{BOW}) was 10 mmHg, as follows:

$$P_{GLO} = \Delta P_F + P_{BOW} + \pi G$$

$$P_{GLO} = (GFR/\ K_{FG}) + 10\ mmHg + [5*(TP/FF*Ln(1/\ -FF)-2)]$$

Finally, in order to calculate renal vascular resistance of the afferent (R_A) and efferent (R_E) renal arteriole, we used the principles of Ohm's law, and the factor 1328 to convert to dyne•sec•cm-5:

$R_A = [(MAP–P_{GLO}/RBF]*1328$

$R_E = [GFR/(K_{FG}*(RBF–GFR)]*1328$

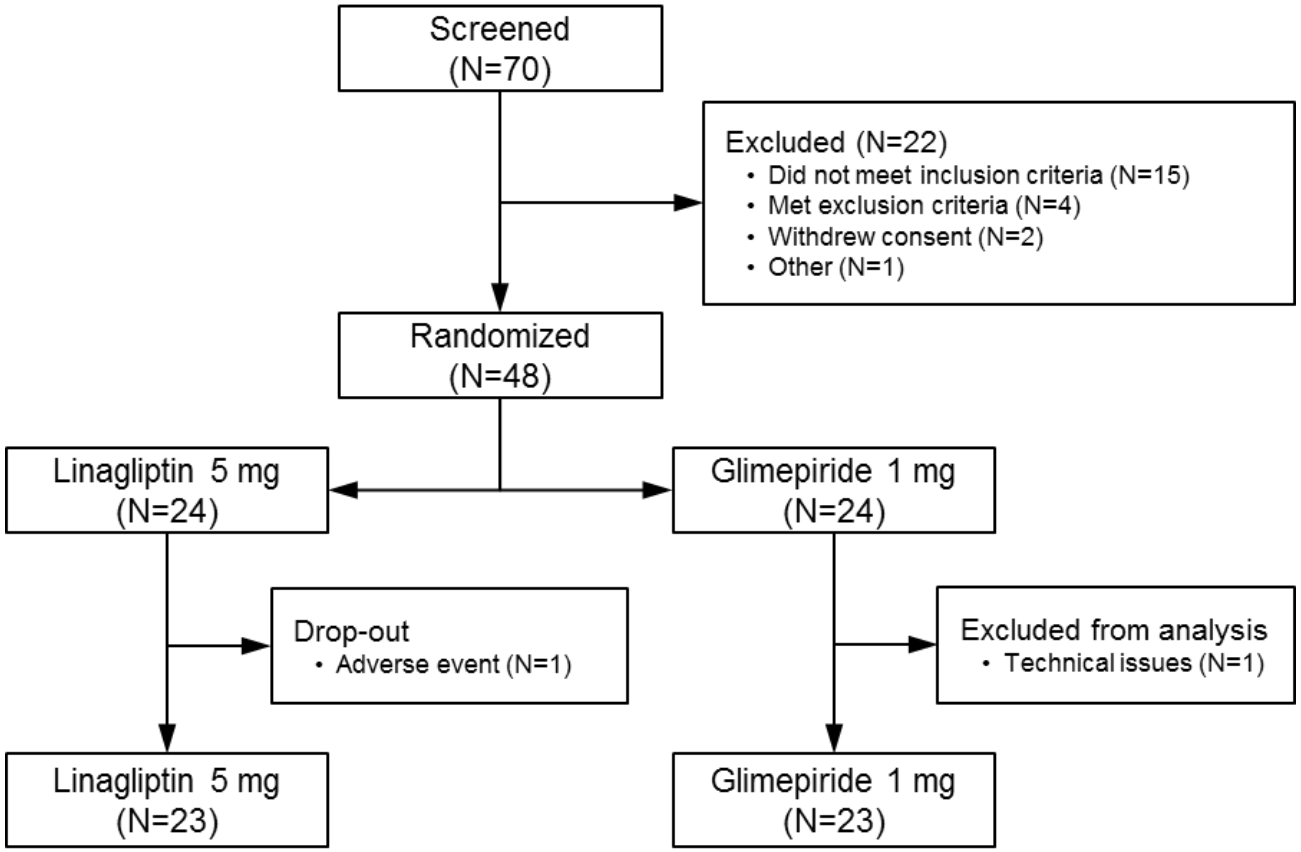
Sample Size Calculation, Data Management and Statistics

At the time of study-design (2013), no randomized controlled trial had been reported to allow evaluation of the effect of DPP-4 inhibition on renal physiology, and therefore, no formal sample-size could be assessed. We calculated that N=21 per treatment-arm should be sufficient to detect a change in GFR of at least 15%, assuming a standard deviation of 10 mL/min, α=0.05 (2-sided testing) and power (1-β) of 80%. To allow for a dropout percentage of 15% and increase power, we decided to include 24 patients per treatment-arm. This was calculated using SAS-software (v.9.2, Cary, NC).

Data were double-entered in an electronic data management system (OpenClinica LLC, version 3.6, Waltham, MA) and transferred to the study database. Before deblinding, inulin-extraction ratios were inspected and urine collections periods characterized by profound collection errors (defined as an inulin extraction-ratio of ≥1.5SD of the mean, or >20% deviation in inulin-extraction ratios before and after treatment) were discarded from the analyses. Urine-collection errors were present in 18 patients (8 randomized to linagliptin and 10 to glimepiride), in whom we calculated GFR and ERPF according to the continuous infusion-method.

Statistical analyses were performed in the per protocol population using SPSS 22.0 (IBM SPSS Inc., Chicago, IL). Multivariable linear regression models were used to examine linagliptin-induced effects compared to glimepiride. Corresponding baseline-values were added as independent variable, to correct for potential between-group differences at baseline. Paired t-tests (Gaussian distributed data) or Wilcoxon signed rank tests (non-Gaussian distributed data) were carried out for within-group comparisons. Spearman correlation analyses were performed to explore associations between changes in renal physiology and exploratory factors deemed relevant. Significance was considered at a two-sided α-level of 0.05. Data are presented as mean±SEM, median [interquartile range] or mean-difference (two-sided 95% confidence interval), unless stated otherwise.

Appendix S2│ Flow diagram of study participants

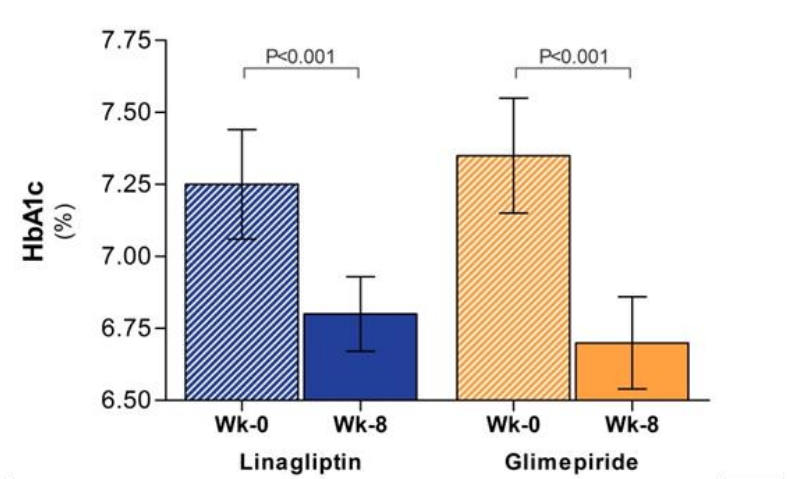


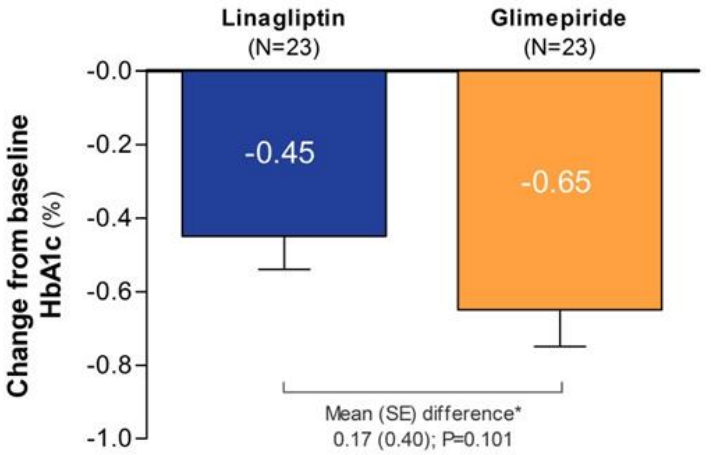
Appendix S3| Demographic and baseline characteristics in the per protocol population

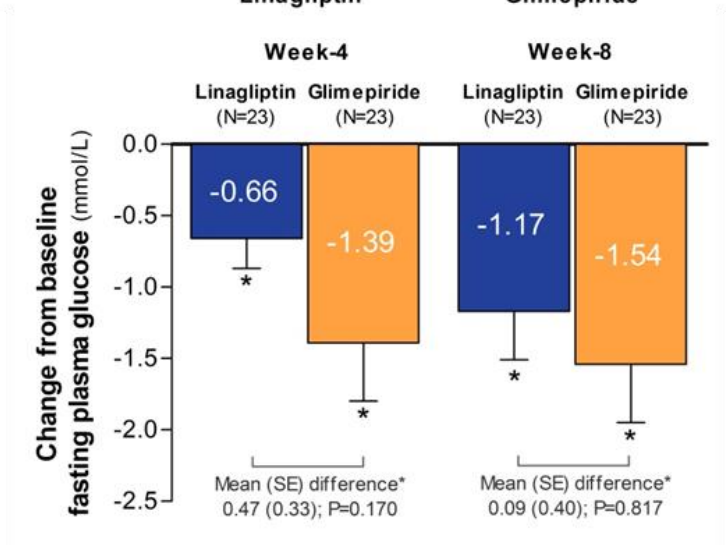
Variables	Linagliptin (N=23)	Glimepiride (N=23)	*P*-value
Age, *years*	62.4 ±9.2	63.5 ±7.9	0.667
Male, *n* (%)	20 (87.0)	18 (78.3)	0.437
Current smoker, *n* (%)	5 (21.7)	5 (21.7)	1.000
Diabetes duration, *years*	7.6 ±4.1	6.4 ±5.3	0.388
Bodyweight, *kg*	101.5 ±16.1	95.0 ±14.5	0.153
Body mass index, *kg/m²*	31.3 ±4.2	30.1 ±3.5	0.289
Systolic blood pressure, *mmHg*	137 ±14	138 ±12	0.800
Diastolic blood pressure, *mmHg*	80 ±9	83 ±8	0.196
Mean arterial pressure, *mmHg*	100 ±9	103 ±8	0.379
Heart Rate, *beats/minute*	63 ±11	66 ±10	0.258
HbA_{1c}, *%*	7.0 [6.6-7.6]	7.0 [6.7-7.7]	0.684
HbA_{1c}, *mmol/mol*	53 [49-60]	53 [50-61]	0.684
Fasting plasma glucose, *mmol/L*	7.90 [7.30-9.20]	8.50 [7.00-9.80]	0.553
eGFR-MDRD, *mL/min/1.73m²*	95.5 ±17.2	91.3 ±13.3	0.355
Albumin-creatinine ratio, *mg/mmol*	0.80 [0.49-3.60]	1.11 [0.47-3.71]	0.852
Microalbuminuria*, *n (%)*	7 (30.4)	7 (30.4)	1.000
Metformin dose, *mg*	1748 ±764	1696 ±726	0.814
Antihypertensive medication use, *n (%)*	16 (69.6)	12 (52.2)	0.227
RAS inhibitor use, *n (%)*	16 (69.6)	11 (47.8)	0.134
ACE-inhibitor use, *n (%)*	8 (34.8)	6 (26.1)	0.522
ARB use, *n (%)*	8 (34.8)	5 (21.7)	0.326
Statin use, *n (%)*	17 (73.9)	12 (52.2)	0.127
Aspirin use, *n (%)*	5 (21.7)	2 (8.7)	0.218

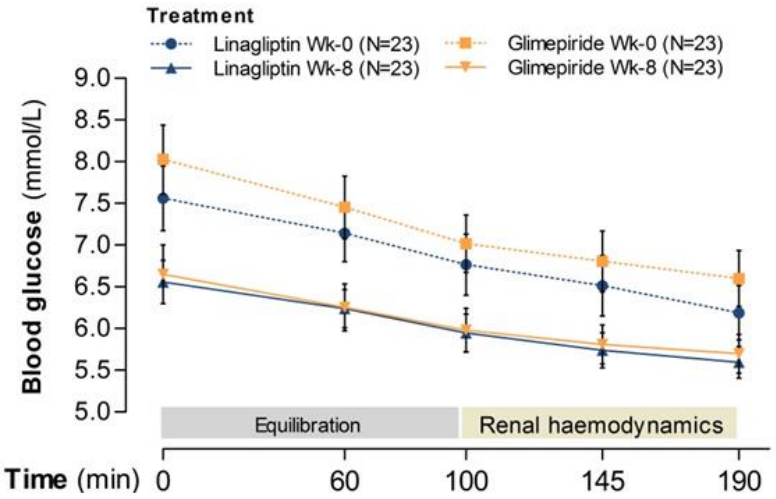
Mean ± SD or median [IQR], unless stated otherwise. Unpaired t-tests or Mann-Whitney tests were used for between-group comparisons. *Defined as a urinary albumin-creatinine ratio ≥3 mg/mmol. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ARB, angiotensin-II receptor blocker; eGFR, estimated glomerular filtration rate; HbA_{1c}, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; MDRD, Modification of Diet in Renal Disease; RAS, renin-angiotensin-system.

Appendix S4| Blood glucose levels during the renal testing procedures







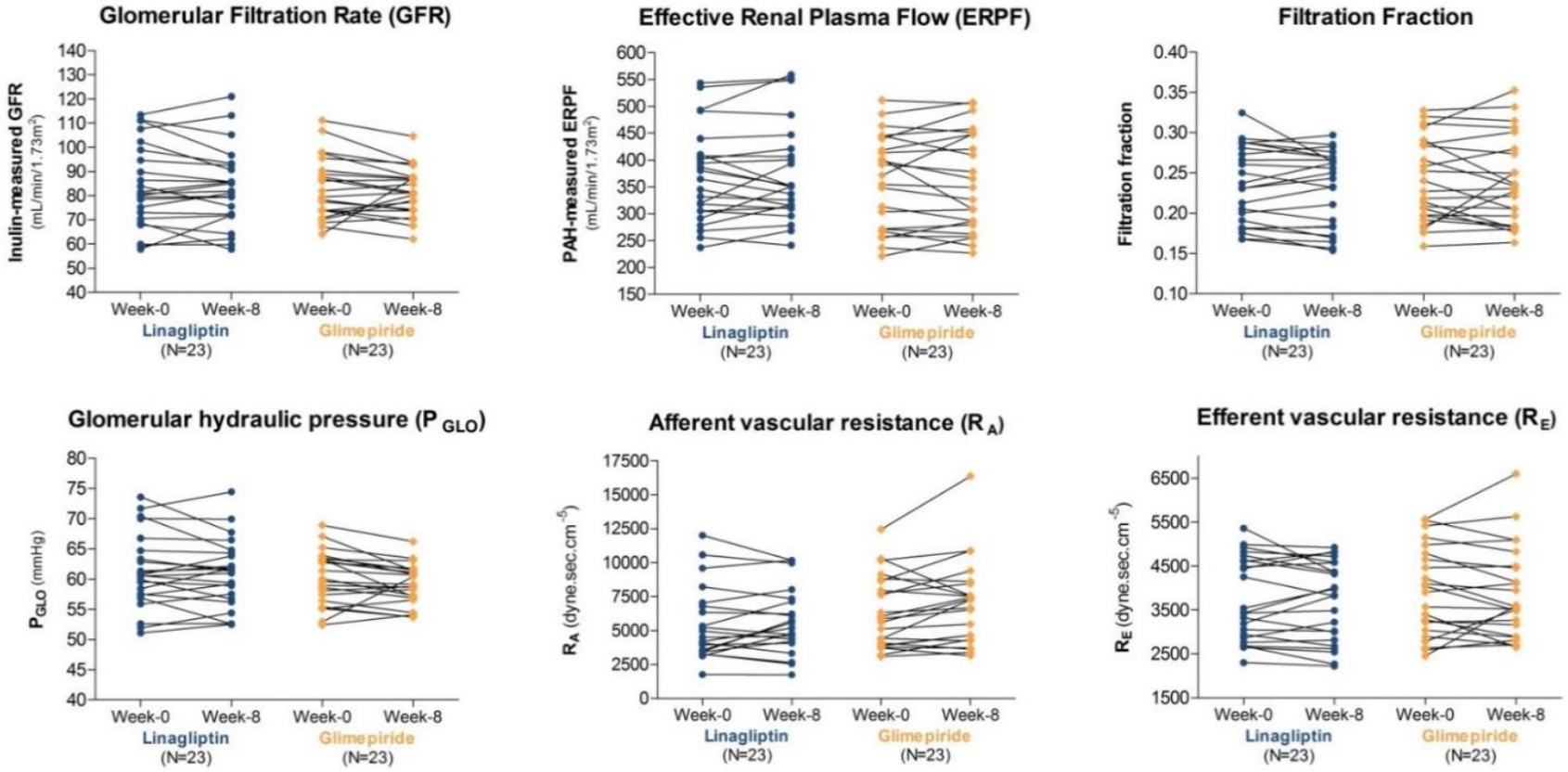


Appendix S5.A│(Intra-)renal hemodynamic responses

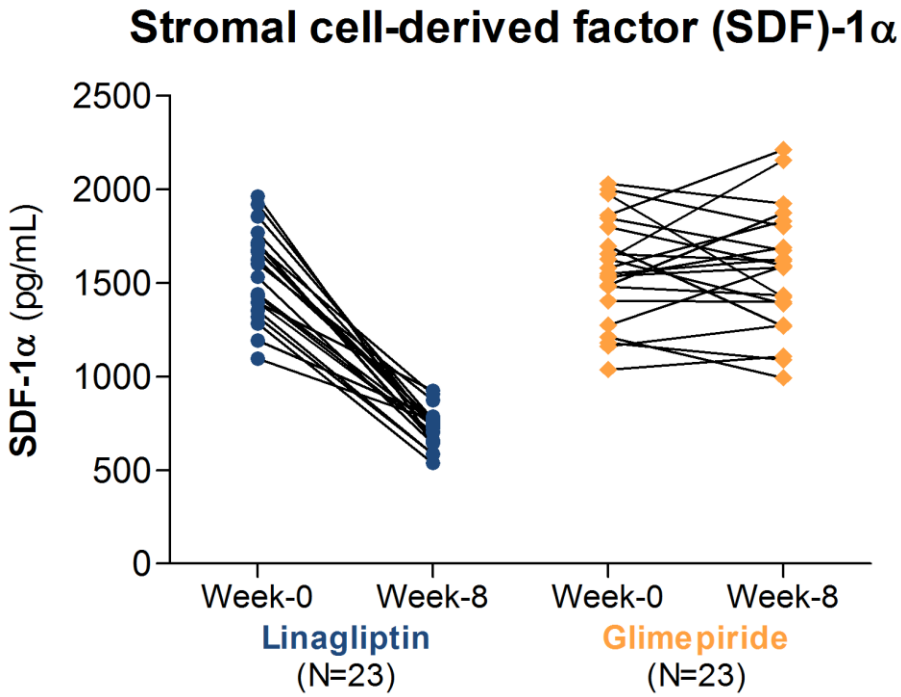
	Linagliptin 5mg QD (N=23)			Glimepiride 1mg QD (N=23)			Mean (95% CI) difference Linagliptin-Glimepiride	
Variables	**Baseline**	**Week-8**	**Within-group**	**Baseline**	**Week-8**	**Within-group**		
Measured renal hemodynamics								
GFR, mL/min/1.73m^2	84.0 ±3.6	83.2 ±3.4	0.547	82.7 ±2.7	81.6 ±2.1	0.471	0.6 (-3.2 to 4.4)	0.760
ERPF, mL/min/1.73m^2	369.6 ±18.3	374.5 ±19.0	0.485	360.2 ±18.2	359.4 ±19.2	0.915	4.5 (-7.7 to 16.8)	0.562
RBF, mL/min/1.73m^2	652.3 ±35.4	652.5 ±34.9	0.987	624.1 ±32.2	623.4 ±33.7	0.961	2.6 (-34.8 to 40.0)	0.891
FF, %	23.1 [18.2-28.1]	24.3 [18.3-26.9]	0.199	22.7 [19.6-28.6]	23.1 [18.4-28.0]	0.834	0.4 (-1.8 to 1.0)	0.543
RVR, mmHg/L/min	0.16 [0.13-0.19]	0.16 [0.13-0.19]	0.605	0.16 [0.13-0.23]	0.18 [0.13-0.22]	0.144	-0.01 (-0.02 to 0.01)	0.491
Estimated intra-renal hemodynamics								
P_{GLO}, mmHg	61.2 ±1.3	60.9 ±1.2	0.561	60.2 ±0.9	59.1 ±0.7	0.064	1.0 (-0.34 to 2.39)	0.136
R_A, dyne.sec.cm^{-5}	4495 [3437-6911]	5181 [4134-7232]	0.375	5874 [3880-8649]	7272 [4340-8445]	**0.033**	-467 (-1259 to 325)	0.241
R_E, dyne.sec.cm^{-5}	3434 [2863-4626]	3831 [2818-4577]	0.523	3905 [3039-4785]	3570 [2889-4497]	0.484	-32 (-290 to 226)	0.804

Data are mean ±SEM, median [IQR] or baseline-corrected mean difference (95% confidence interval; CI) using multiple linear regression to examine baseline-corrected linagliptin-induced effects compared to glimepiride. Paired t-tests or Wilcoxon signed rank 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; P_{GLO}, glomerular hydraulic pressure; R_A, afferent renal arteriolar resistance; RBF, renal blood flow; R_E, efferent renal arteriolar resistance; RVR, renal vascular resistance.

Appendix S5.B│Individual (intra) renal hemodynamic responses following linagliptin or glimepiride



Appendix S5.C│Individual responses in stromal cell-derived factor-1a following linagliptin or glimepiride



Appendix S6│Responses in electrolytes, metabolic variables and anthropometrics following linagliptin or glimepiride

Variables	Linagliptin 5mg QD (N=23)			Glimepiride 1mg QD (N=23)			Mean (95% CI) difference Linagliptin-Glimepiride	
	Baseline	Week-8	Within-group P-value	Baseline	Week-8	Within-group P-value		
Plasma electrolytes								
Sodium, mmol/L	138.7 ±0.5	139.0 ±0.4	0.660	138.2 ±0.4	139.9 ±0.5	0.001	-1.2 (-2.4 to -0.1)	0.037
Potassium, mmol/L	4.1 ±0.1	4.3 ±0.1	0.012	4.0 ±0.1	4.1 ±0.1	0.325	0.1 (-0.0 to 0.3)	0.139
Urea, mmol/L	5.0 ±0.2	5.6 ±0.2	0.021	4.7 ±0.2	5.1 ±0.3	0.157	0.2 (-0.4 to 0.9)	0.500
Metabolic variables & biomarkers								
Total cholesterol, mmol/L	3.6 [3.3-4.3]	3.7 [3.2-3.9]	0.352	4.6 [4.0-5.3]	4.7 [3.9-5.3]	0.509	-0.1 (-0.3 to 0.1)	0.288
HDL-cholesterol, mmol/L	0.97 [0.93-1.18]	1.00 [0.95-1.15]	0.616	1.19 [0.93-1.31]	1.12 [0.91-1.31]	0.314	0.01 (-0.05 to 0.07)	0.713
LDL-cholesterol, mmol/L	1.80 [1.70-2.50]	2.00 [1.60-2.40]	0.178	2.35 [2.18-3.00]	2.40 [2.20-3.20]	0.359	-0.20 (0.40 to 0.00)	*0.053*
Triglycerides, mmol/L	1.6 ±0.1	1.5 ±0.1	0.569	2.3 ±0.2	2.1 ±0.2	0.063	0.1 (-0.2 to 0.5)	0.334
Albumin, g/L	37.2 ±0.4	37.3 ±0.4	0.902	36.4 ±0.5	35.7 ±0.4	**0.005**	1.0 (0.2 to 1.8)	**0.013**
AST, U/L	21 [18-25]	21 [19-25]	0.308	23 [18-26]	24 [19-28]	0.423	-1 (-4 to 2)	0.624
ALT, U/L	25 [22-35]	25 [18-31]	0.423	28 [19-38]	27 [21-34]	0.542	-1 (-4 to 3)	0.684
Amylase, U/L	49 [31-62]	50.0 [34.0-70.0]	**0.004**	45 [35-54]	50 [40-62]	**0.011**	1.00 (0.93 to 1.07)$	0.971
Lipase, U/L	37 [29-50]	42.0 [30.0-61.0]	0.088	34 [28-42]	41 [25-57]	0.124	1.02 (0.87 to 1.17)$	0.771
Body weight and composition								
Hip circumference, cm	111.4 ±1.6	111.8 ±1.8	0.407	107.9 ±1.2	108.3 ±1.2	0.433	0.0 (-1.4 to 1.4)	0.974
Waist-hip ratio	1.02 ±0.1	1.02 ±1.01	0.671	1.02 ±0.02	1.03 ±0.02	0.283	-0.00 (-0.02 to 0.01)	0.910
Body fat, %	33.9 ±1.4	33.6 ±1.4	0.295	33.5 ±1.1	33.7 ±1.2	0.574	-0.6 (-1.5 to 0.4)	0.244

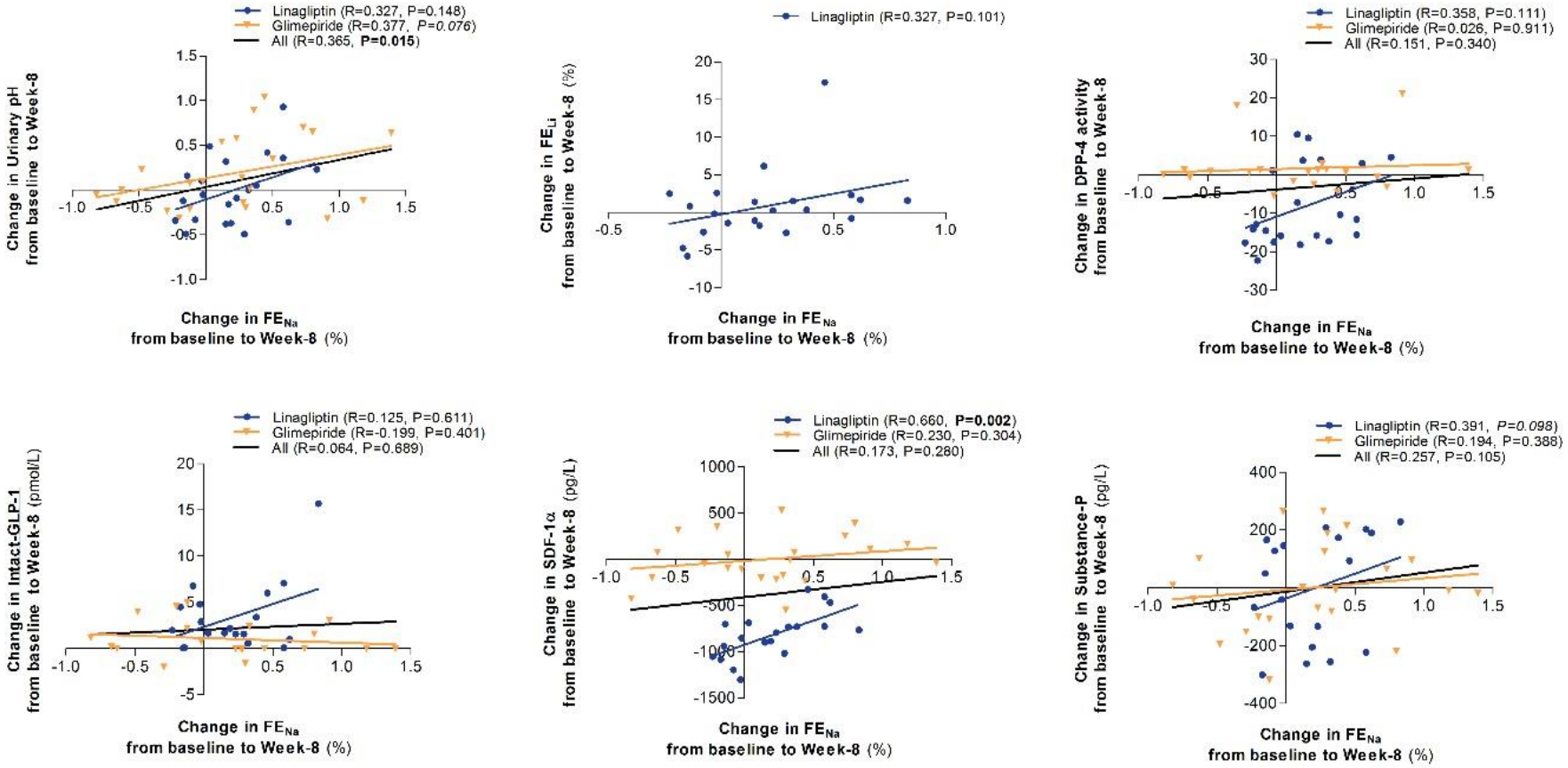
Mean ±SEM, median [IQR] or baseline-corrected mean difference (95% confidence interval; CI) using multiple linear regression to examine baseline-corrected linagliptin-induced effects compared to glimepiride. Paired t-tests or Wilcoxon signed rank tests were used for within-group comparisons. $ Indicates baseline-corrected ratio using multiple linear regression. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Appendix S7│Exploratory correlation analyses between change in fractional urinary sodium excretion and change in selected natriuretic factors from baseline to Week-8

Characteristic	Delta-FE_{Na}		
	All	**Linagliptin**	**Glimepiride**
Delta-**Urinary pH**	**0.365** **P=0.015**	0.327 P=0.148	0.377 *P=0.076*
Delta-FE_{Li}	NA	0.327 P=0.101	NA
Delta-**Systolic BP**, mmHg	0.249 P=0.112	0.035 P=0.886	0.342 P=0.110
Delta-**GFR**, mL/min/1.73m^2	-0.184 P=0.231	-0.253 P=0.268	-0.286 P=0.187
Delta-**Fasting insulin**	0.138 P=0.376	0.323 P=0.164	0.034 P=0.879
Delta-**Fasting glucagon**	0.132 P=0.412	-0.339 P=0.156	0.036 P=0.875
Delta-**DPP-4 activity**, RLA	0.151 P=0.340	0.358 P=0.111	0.026 P=0.911
Delta-**Total GLP-1**, kg	-0.052 P=0.747	-0.083 P=0.736	0.029 P=0.896
Delta-**Intact GLP-1**, mmHg	0.064 P=0.689	0.125 P=0.611	-0.199 P=0.401
Delta-**SDF-1α**, pg/L	0.173 P=0.280	**0.660** **P=0.002**	0.230 P=0.304
Delta-**Active NPY**, mmol/L	-0.130 P=0.424	0.026 P=0.915	-0.240 P=0.294
Delta-**Pro NPY**, µmol/L	0.061 P=0.709	-0.0.91 P=0.710	-0.121 P=0.602
Delta-**Substance P**, pg/L	0.257 P=0.105	0.391 *P=0.098*	0.194 P=0.388

Data are presented as Spearman correlation coefficients and corresponding P-value. Abbreviations: BP, blood pressure; DPP-, dipeptidyl-peptidase; FE, fractional excretion; GFR, glomerular filtration rate; GLP-1, glucagon-like peptide-1; NPY, Neuropeptide Y; RLA, relative luciferase activity, SDF-1, stromal cell-derived factor 1.

Appendix S8│ Exploratory correlation analyses between change in FE_{Na} and change in selected natriuretic factors from baseline to Week-8



Data are presented as Spearman correlation coefficients and corresponding P-value. Abbreviations: DPP, dipeptidyl-peptidase; FE, fractional excretion; GLP-1, glucagon-like peptide-1; SDF-1α, stromal cell-derived factor-1α

Appendix S9│Number (%) of patients with ≥1 event during the randomized period

Adverse events	Linagliptin 5 mg (N=24)	Glimepiride 1 mg (N=24)	P-value
Overall			
Any adverse event	12 (50%)	15 (63%)	0.383
Serious adverse event	0 (0%)	0 (0%)	1.000
Adverse event of special interest	1 (4%)	1 (4%)	1.000
Adverse event leading to discontinuation	0 (0%)	0 (0%)	1.000
Deaths	0 (0%)	0 (0%)	1.000
Specific adverse events			
Probable symptomatic hypoglycemia*	1 (4%)	6 (25%)	**0.041**
Upper respiratory tract infection	2 (8%)	3 (13%)	0.637
Cough	1 (4%)	1 (4%)	1.000
Xerostomia	1 (4%)	1 (4%)	1.000
Diarrhea	2 (8%)	4 (17%)	0.383
Nausea	1 (4%)	1 (4%)	1.000
Pyrosis	1 (4%)	0 (0%)	0.312
Atrial fibrillation	1 (4%)	0 (0%)	0.312
Dizziness	0 (0%)	1 (4%)	0.312
Chest complaints e.c.i.	1 (4%)	0 (0%)	0.312
Malaise	1 (4%)	0 (0%)	0.312
Headache	1 (4%)	2 (8%)	0.551
Arthralgia / Back pain	1 (4%)	2 (8%)	0.551
Generalized pruritus / urticaria	1 (4%)	1 (4%)	1.000
Purpura	0 (0%)	1 (4%)	0.312
Polyuria / Polydipsia	0 (0%)	1 (4%)	0.312
Dental infection	0 (0%)	1 (4%)	0.312
Total number of adverse events	15	25	

*An event during which symptoms of hypoglycemia are not accompanied by a plasma glucose determination, but that was presumably caused by a plasma glucose concentration ≤3.9 mmol/L.