Postprandial renal haemodynamic effects of the dipeptidyl peptidase-4 inhibitor linagliptin versus the sulphonylurea glimepiride in adults with type 2 diabetes (RENASLIS): A predefined substudy of a randomized, double-blind trial

Marcel H. A. Muskiet MD1 | Lennart Tonneijck MD1 | Mark M. Smits MD1 | Mark H. H. Kramer MD1 | D. Margriet Ouwens PhD2,3,4 | Bolette Hartmann PhD5 | Jens J. Holst PhD5 | A. H. Jan Danser MD6 | Jaap A. Joles DVM7 | Daniël H. van Raalte MD1

Abstract

Aim: To determine the effect of the dipeptidyl peptidase-4 inhibitor linagliptin on postprandial glomerular hyperfiltration compared with the sulphonylurea glimepiride in adults with type 2 diabetes (T2D).

Materials and Methods: In this predefined substudy within a randomized, double-blind, parallel-group, intervention trial, overweight people with T2D without renal impairment were treated with once-daily linagliptin 5 mg (N = 10) or glimepiride 1 mg (N = 13) as an add-on to metformin for 8 weeks. After a standardized liquid protein-rich meal, the glomerular filtration rate (GFR) and effective renal plasma flow were determined by inulin and para-aminohippuric acid clearance, respectively, based on timed urine sampling. Intrarenal haemodynamics were estimated using the Gomez equations. Glucoregulatory/vasoactive hormones, urinary pH and fractional excretions (FE) of sodium, potassium and urea were measured.

Results: Compared with glimepiride, linagliptin increased the postprandial filtration fraction (FF; mean difference 2.1%-point; P = .016) and estimated glomerular hydraulic pressure (mean difference 3.0 mmHg; P = .050), and tended to increase GFR (P = .08) and estimated efferent renal arteriolar resistance (RE; P = .08) from baseline to week 8. No differences in FE were noted. Glimepiride reduced HbA1c more than linagliptin (mean difference −0.40%; P = .004), without between-group differences in time-averaged postprandial glucose levels. In the linagliptin group, change in FF correlated with change in mean arterial pressure (R = 0.807; P = .004) and time-averaged mean glucagon (R = 0.782; P = .008), but not with changes in glucose, insulin, intact glucagon-like peptide-1, renin or FENa. Change in glucagon was associated with change in RE (R = 0.830; P = .003).

Received: 19 May 2021 Revised: 31 August 2021 Accepted: 17 September 2021
DOI: 10.1111/dom.14557

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2021 The Authors. Diabetes, Obesity and Metabolism published by John Wiley & Sons Ltd.
**1 | INTRODUCTION**

A single, particularly protein-rich meal increases the glomerular filtration rate (GFR) by ~40%-100%, a phenomenon known as ‘postprandial hyperfiltration’.\(^1\)\(^2\) This renal haemodynamic response to nutrient-ingestion peaks after 60-120 minutes, lasts a few hours, and is independent of arterial pressure.\(^1\)\(^2\) Postprandial hyperfiltration, and the consequent rise in filtered load of circulating solutes, is part of the physiological ‘gut-renal axis’, i.e. a rapid-acting feed-forward loop that regulates postprandial fluid electrolyte homeostasis.\(^3\) It is believed to mitigate a rise in potentially injurious gut-absorbed solutes and catabolic wastes, and accelerate their excretion. Mediators that may underlie postprandial hyperfiltration include the effects of absorbed glucose and amino acids, vasoactive agents, neuronal pathways, and intrarenal mechanisms (e.g. tubuloglomerular feedback [TGF]).\(^1\) In adults with type 2 diabetes (T2D), hyperfiltration is considered an independent renal risk factor, predisposing to irreversible nephron damage by, among others, imposing physical stress on the filtration barrier and increasing renal oxygen demand to drive tubular reabsorption.\(^1\)\(^3\) Mechanistic understanding of postprandial renal haemodynamics in T2D, and assessment of treatment-induced effects on this response, may aid to reduce renal risk and improve long-term kidney outcome.\(^5\)

Glucagon-like peptide-1 (GLP-1) is a gut-derived hormone, released within minutes of food intake, which principally regulates glucose metabolism by potentiation of insulin secretion, inhibition of glucagon release, and deceleration of the gastric emptying rate.\(^3\) For management of hyperglycaemia in T2D, two classes of incretin-based therapies were introduced: GLP-1 receptor agonists (RAs) and dipeptidyl peptidase-4 inhibitors (DPP-4is), which prevent the degradation/inactivation of native peptides, such as GLP-1.\(^3\) Considerable interest exists in identifying the effects of these drugs beyond glucose lowering, with several landmark trials suggesting modest glucose-independent benefits on renal endpoints.\(^2\)\(^3\)\(^9\)\(^10\)\(^12\)\(^13\) Interestingly, GLP-1 is implicated as a mediator in the gut-renal axis, with proposed direct (GLP-1-R–mediated) and indirect (e.g. via the renin angiotensin system [RAS]) actions on the kidney.\(^3\) Acute GLP-1RA administration increased GFR and natriuresis in healthy rodents and humans.\(^7\)\(^8\) In people with T2D, acute and prolonged GLP-1RA\(^5\)\(^-\)\(^12\) or DPP-4i therapy\(^12\)\(^-\)\(^15\) did not affect fasting renal haemodynamics, but increased urinary sodium excretion. In the postprandial state, in which humans spend most of their day, data on the renal effects of these therapies are scarce. We previously hypothesized that GLP-1RAs and DPP-4is may reduce postprandial hyperfiltration in T2D.\(^3\) Although prolonged treatment with the GLP-1RA lixisenatide had a sustained natriuretic effect after meal ingestion, the drug did not affect postprandial (intra) renal haemodynamics compared with insulin glulisine in adults with T2D.\(^16\) No studies have explored the effect of DPP-4is on postprandial renal physiology. To identify drug-specific properties and allow clinically relevant comparisons, a sulphonylurea was selected as an active control. We hypothesize that the DPP-4i linagliptin reduces postprandial GFR, effective renal plasma flow (ERPF), and estimated glomerular hydraulic pressure (P\(_{\text{GLO}}\)) compared with the sulphonylurea glimepiride in patients with T2D by activating TGF (via proximal natriuresis and/or glucagon-suppression), reducing RAS activity, and increasing the circulating levels of active GLP-1.

**2 | MATERIALS AND METHODS**

**2.1 | Trial design and population**

This is a predefined substudy of the RENALIS trial, a phase IV, randomized, double-blind, comparator-controlled, parallel-group, mechanistic intervention trial, conducted from May 2014 to April 2016 at the Amsterdam University Medical Centers, location VUMC, Amsterdam, The Netherlands. The co-primary objective of the main trial was to assess the effect of 8 weeks of treatment with linagliptin versus glimepiride on the change in fasting renal haemodynamics (i.e. measured GFR and ERPF), the results of which have been published.\(^1\)\(^3\) Following a study amendment (February 2015), renal haemodynamics were also measured in the postprandial state in the final 26 patients enrolled in RENALIS. The results of this substudy are described here. The local institutional review board, ethics committee, and competent local authorities approved the trial protocol and its amendments. The trial complied with the Declaration of Helsinki and Good Clinical Practice, and was registered with ClinicalTrials.gov (ID: NCT02106104). Written informed consent was obtained prior to any trial-related activities.

The eligibility criteria were described previously.\(^1\)\(^5\) In brief, all patients were Caucasian, men or postmenopausal women, aged 35-75 years, had T2D, received metformin monotherapy, had an HbA1c of 6.5%-9.0% (48-75 mmol/mol), and a body mass index of 25 kg/m\(^2\) or higher. In cases of hypertension and/or albuminuria, treatment included a RAS blocker for 3 months or longer. The main exclusion criteria were history of pancreatic, active liver or malignant

**Conclusions:** In contrast to our hypothesis, compared with glimepiride, linagliptin does not reduce postprandial hyperfiltration, yet appears to increase FF after meal ingestion by increasing blood pressure or R\(_E\).

**KEYWORDS**
dipeptidyl peptidase-4, DPP-4 inhibitor, glomerular filtration rate, postrandial hyperfiltration, sulphonylurea, type 2 diabetes
disease, an estimated GFR of less than 60 mL/min/1.73m², 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.

2.2 | Intervention and randomization

Following baseline measurements, patients were randomized 1:1 (block size 4; performed by an independent trial pharmacist using computer-generated numbers) to receive once-daily linagliptin 5 mg or glimepiride 1 mg, added to ongoing metformin (dose unchanged throughout the study) for 8 weeks (Appendix S1A). Patients were instructed to take their study drug daily at the same time in the evening. The study drugs were overencapsulated, producing visually identical oral capsules by an independent Good Medical Practice-certified clinical research organization (ACE-Pharmaceutical, Zeewolde, The Netherlands); patients and investigators remained blinded to treatment status until database unlock.

2.3 | Study endpoints

The predefined co-primary endpoint of this substudy was linagliptin-induced change in postprandial GFR and ERPF from baseline to week 8, compared with glimepiride, as derived from inulin and para-aminohippuric acid (PAH) clearances. All other (intra)renal variables, tubular functions, and blood pressure were considered secondary endpoints. Changes in bodyweight, haematocrit, body water percentage, HbA1c, blood glucose, plasma renin concentration (PRC), insulin, glucagon, GLP-1 and DPP-4 activity were analysed as safety and/or exploratory endpoints.

2.4 | Study protocol

The protocol for determination of the fasting study endpoints in the main study was published previously. In the current substudy, following fasting measurements, patients received a liquid mixed meal, after which renal haemodynamics and other endpoints were assessed at baseline and after 8 weeks of therapy (Appendix S1A,B). In brief, after an overnight fast, fasting GFR and ERPF were determined by the standard renal clearance technique based on timed urine sampling (main study; Appendix S1B). Diuresis was prompted by oral intake of 10 mL/kg (maximum 1000 mL) of tap water during the 90-minute inulin/PAH-equilibration period, followed by an intake of 200 mL/h. Following assessment of the fasting renal haemodynamics, the 26 patients enrolled in this substudy received a 400-mL liquid meal (Nutridrink Yoghurt Style, Nutricia; Appendix S2), during which the infusion of inulin/PAH was continued. Forty-five minutes after the meal, patients emptied their bladders to achieve a zero point for clearance determination; urine was then collected by spontaneous voiding for two 45-minute periods to assess postprandial renal haemodynamics (Appendix S1B). Aliquots were drawn from each collection and analysed 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. Haematocrit was determined at the midpoint of the two postprandial urine collection periods. Blood was taken for PRC after 30 minutes of supine rest. Blood samples to determine explanatory metabolic variables (e.g. glucose, insulin, GLP-1, and glucagon) were taken before and at preset intervals following meal ingestion (up to 135 minutes postmeal). Details of the assays used are described in Appendix S3. For this analysis, systolic and diastolic blood pressure, mean arterial pressure (MAP) and heart rate were measured on arrival at the Clinical Research Unit (~3 hours before meal ingestion), and 85 minutes postmeal, by an automated oscillometric device (Dinamap, GE Healthcare, Little Chalfont, UK).

2.5 | Calculation of renal endpoints

GFR and ERPF were calculated from inulin and PAH clearances, respectively, based on timed urine sampling and averaged from the two consecutive postprandial urine collection periods. Renal blood flow (RBF) was calculated as ERPF/(1 – haematocrit), filtration fraction (FF) as GFR/ERPF, and renal vascular resistance as MAP/RBF. Intrarenal haemodynamic functions (i.e. PGLO and afferent and efferent arteriolar resistances [RA and RE, respectively]) were estimated according to the Gomez equations (Appendix S4). Fractional sodium (FENa), potassium (FEK), and urea (FEU) excretion were calculated using inulin as the reference substance. Renal haemodynamic variables were corrected for body surface area using the Mosteller formula.

2.6 | Sample size calculation, data management, and statistics

Because no randomized controlled trial had evaluated the effect of DPP-4 inhibition on postprandial renal physiology at the time of study design and amendment, no formal sample size was assessed for this substudy. 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 collection periods characterized by profound collection errors (defined as an inulin extraction ratio of ≥1.5 SD 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 three patients, all randomized to linagliptin. Statistical analyses were performed using SPSS 22.0 (IBM SPSS Inc., Chicago, IL). Multivariable linear regression models were used to examine linagliptin-induced effects compared with glimepiride; corresponding baseline values were added as an independent variable, to correct for potential between-group baseline differences. 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 relevant exploratory factors. Significance was considered at a two-sided $\alpha$-level of .05 or less. Data are presented as mean ± SEM, median [interquartile range], or mean difference (two-sided 95% confidence interval), unless stated otherwise.

3 | RESULTS

A total of 70 patients were screened, 48 were included and randomized in the main trial, of which 26 were included in the current substudy (Appendix S5). The demographic and clinical characteristics of the analysed 23 patients were generally well balanced across treatment groups (Table 1). Notably, there were more female patients randomized to glimepiride compared with linagliptin (four [31%] vs. no [0%] females), which may explain baseline between-group differences in bodyweight.

3.1 | Glycaemic control

Mean changes in HbA1c from baseline to week 8 were $-0.40\% \pm 0.11\%$ for linagliptin and $-0.80\% \pm 0.16\%$ for glimepiride; glimepiride was superior to linagliptin in reducing HbA1c (between-group mean difference $0.40\% [95\% CI 0.14\%$ to $0.64\%; P = .004$]; Appendix S6). Blood glucose increased following meal ingestion without any between-group differences (Figure 1; Appendix S6). After 8 weeks, time-averaged mean (TAM) postprandial glucose during the renal tests was similarly reduced from baseline (between-group mean difference $0.12 \text{ mmol/L} [-0.74 \text{ to } 0.98; P = .774]$; Appendix S6).

| TABLE 1 | Demographic and baseline clinical characteristics in the per protocol population |
|---------|---------------------------------|
| Variables | Linagliptin (N = 10) | Glimepiride (N = 13) | $P$ value |
| Age, y | 62.3 ± 7.7 | 66.4 ± 6.4 | .174 |
| Male, n (%) | 10 (100) | 9 (69.2) | .104 |
| Current smoker, n (%) | 2 (20.0) | 2 (15.4) | .903 |
| Diabetes duration, y | 7.1 ± 4.5 | 7.5 ± 5.5 | .841 |
| Bodyweight, kg | 109.0 ± 11.4 | 92.8 ± 15.0 | .010 |
| Body mass index, kg/m² | 32.2 ± 3.9 | 30.4 ± 3.9 | .289 |
| Systolic blood pressure, mmHg | 135 ± 13 | 135 ± 12 | .955 |
| Diastolic blood pressure, mmHg | 81 ± 10 | 80 ± 6 | .892 |
| Mean arterial pressure, mmHg | 100 ± 9 | 100 ± 7 | .974 |
| Heart rate, beats/min | 61 ± 9 | 65 ± 10 | .327 |
| HbA1c, % | 7.35 ± 1.02 | 7.35 ± 1.16 | .992 |
| HbA1c, mmol/mol | 56.9 ± 11.2 | 56.8 ± 12.6 | .993 |
| Fasting plasma glucose, mmol/L | 7.95 [7.55-10.53] | 8.90 [7.70-10.50] | .535 |
| Total cholesterol, mmol/L | 4.02 ± 1.57 | 4.68 ± 0.87 | .215 |
| LDL-C, mmol/L | 2.27 ± 1.15 | 2.55 ± 0.78 | .488 |
| HDL-C, mmol/L | 1.04 ± 0.16 | 1.18 ± 0.32 | .218 |
| Triglycerides, mmol/L | 1.59 ± 0.72 | 2.08 ± 0.84 | .157 |
| Fasting mGFR, mL/min/1.73m² | 90.4 ± 16.6 | 83.5 ± 11.8 | .251 |
| Albumin-creatinine ratio, mg/mmol | 0.61 [0.39-4.39] | 0.89 [0.45-2.53] | .648 |
| Microalbuminuria a, n (%) | 3 (30.0) | 3 (23.1) | 1.000 |
| Metformin dose, mg | 1485 ± 758 | 1577 ± 772 | .778 |
| Antihypertensive medication use, n (%) | 8 (80.0) | 7 (53.8) | .379 |
| RAS inhibitor use, n (%) | 8 (80.0) | 7 (53.8) | .379 |
| ACE-inhibitor use, n (%) | 4 (40.0) | 3 (23.1) | .650 |
| ARB use, n (%) | 4 (40.0) | 4 (30.8) | .685 |
| Statin use, n (%) | 8 (80.0) | 6 (46.2) | .197 |
| Aspirin use, n (%) | 3 (30.0) | 1 (7.7) | .281 |

Note: Data are shown as mean ± SD, median [IQR], or n (%). Unpaired t-tests, Mann–Whitney U or Fisher’s exact tests were used for between-group comparisons.

Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin-II receptor blocker; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; mGFR, inulin-measured glomerular filtration rate; RAS, renin angiotensin system.

aDefined as a urinary albumin-creatinine ratio $\geq 3 \text{ mg/mmol}$. 

118  MUSKIET ET AL.
Overall, these effects satisfied the design objective of glycaemic equipoise during postprandial renal haemodynamics assessments.

3.2 | (Intra)renal haemodynamics and tubular functions

Eight-week administration of linagliptin tended to increase postprandial GFR relative to baseline (7.9 ± 3.8 mL/min/1.73m²; \( P = .068 \)) compared with glimepiride (between-group mean difference 8.6 mL/min/1.73m² [−1.2 to 18.4; \( P = .083 \)]; Table 2, Figure 2; individual responses in Appendix S7). There were no within-group or between-group changes from baseline to week 8 in ERPF or renal vascular resistance. Linagliptin compared with glimepiride increased postprandial FF by 2.1% (0.4% to 3.8%; \( P = .016 \)) and PGLO by 3.0 mmHg (0.0 to 6.0 mmHg; \( P = .050 \)) after 8 weeks. This effect remained fundamentally unchanged in a males-only sensitivity analysis (Appendix S8), or after correction for changes in HbA1c. No significant changes or between-group differences were seen in RA or RE. In this subpopulation, fasting renal haemodynamics were not affected by either treatment, similar to the whole population in the main trial (Appendix S9).

No significant within- or between-group changes from baseline to week 8 were seen in postprandial FENa, FEK, FEurea, urinary pH, and osmolality (Table 2). After 8 weeks, compared with linagliptin, glimepiride increased the postprandial sodium concentration, as well as the changes in potassium and urea concentrations from fasting to postprandial (Appendix S10).

3.3 | Anthrophometrics and systemic haemodynamics

Bodyweight was unaffected by linagliptin, and increased with glimepiride from baseline to week 8, reaching a significant between-group difference (mean difference −1.25 kg: −2.31 to −0.19 kg; \( P = .023 \); Appendix S6). No treatment differences were observed in postprandial systolic or diastolic blood pressure, MAP, or heart rate at 85 minutes postmeal after 8 weeks of treatment relative to baseline. However, in the current subpopulation, as reported previously,20 linagliptin blunted maximum postprandial decrease in systolic blood pressure relative to baseline (from −7.8 ± 2.3 mmHg at baseline, to −0.7 ± 2.3 mmHg at week 8; \( P = .009 \)); this was significant compared with glimepiride (\( P = .010 \)).

3.4 | Hormones

Changes in postprandial hormones are shown in Figure 1 and Appendix S6. There were no within- or between-group differences in

![Figure 1](image-url)
| Variables                                      | Linagliptin 5 mg QD (N = 10) | Glimepiride 1 mg QD (N = 13) | Mean (95% CI) difference Linagliptin-glimepiride |
|------------------------------------------------|------------------------------|-------------------------------|--------------------------------------------------|
|                                                | Baseline                     | Week 8                        | Within-group                                      | Baseline                   | Week 8                  | Within-group                                      |
|                                                | Mean (95% CI) difference     | P value                       |                                                  | Mean (95% CI)              | P value                 |                                                  |
| **Measured renal haemodynamics**               |                              |                               |                                                  |                            |                         |                                                  |
| GFR, mL/min/1.73m²                             | 88.4 ± 5.8                   | 97.3 ± 4.4                    | .068                                             | 89.2 ± 4.6                 | 88.6 ± 3.9              | .884                                             | 8.6 (-1.2 to 18.4)                             | .083 |
| ERPF, mL/min/1.73m²                            | 359.5 ± 24.7                 | 380.3 ± 22.0                  | .157                                             | 368.2 ± 17.5               | 386.3 ± 22.8            | .295                                             | 1.1 (-45.5 to 47.7)                            | .962 |
| RBF, mL/min/1.73m²                             | 628.6 ± 44.1                 | 661.1 ± 37.0                  | .183                                             | 628.0 ± 30.8               | 645.0 ± 40.1            | .570                                             | 15.7 (-64.0 to 95.3)                           | .686 |
| FF, %                                         | 25.0 ± 0.6                   | 25.8 ± 0.7                    | .078                                             | 24.4 ± 0.9                 | 23.3 ± 0.8              | .131                                             | 2.1 (0.4 to 3.8)                               | .016 |
| RVR, mmHg/L/min                                | 0.170 ± 0.015                | 0.153 ± 0.010                 | .573                                             | 0.164 ± 0.009              | 0.163 ± 0.010           | .947                                             | -0.005 (-0.026 to 0.016)                        | .636 |
| **Estimated intrarenal haemodynamics**         |                              |                               |                                                  |                            |                         |                                                  |
| P<sub>GLO</sub>, mmHg                          | 62.7 ± 1.8                   | 64.1 ± 1.5                    | .229                                             | 61.6 ± 1.3                 | 60.4 ± 1.1              | .376                                             | 3.0 (0.0 to 6.0)                               | .050 |
| R<sub>A</sub>, dyne.s.cm<sup>-5</sup>         | 4654 [3769-6312]             | 3804 [3186-6037]              | .314                                             | 4765 [3850-6141]           | 5141 [4373-6445]        | .600                                             | -519 (-1647 to 609)                            | .348 |
| R<sub>E</sub>, dyne.s.cm<sup>-5</sup>         | 3887 [3735-4361]             | 4189 [3880-4597]              | .074                                             | 4016 [3867-4206]           | 3952 [3577-4322]        | .463                                             | 296 (-39 to 631)                               | .080 |
| **Tubular functions**                          |                              |                               |                                                  |                            |                         |                                                  |
| Fasting FE<sub>Na</sub>, %                     | 1.11 ± 0.15                  | 1.24 ± 0.19                   | .160                                             | 1.15 ± 0.14                | 1.46 ± 0.24             | .119                                             | -0.17 (-0.65 to 0.30)                          | .456 |
| Postprandial FE<sub>Na</sub>, %                | 1.14 ± 0.12                  | 1.09 ± 0.16                   | .623                                             | 0.90 ± 0.12                | 1.09 ± 0.07             | .055                                             | -0.15 (-0.40 to 0.11)                          | .234 |
| ΔPP-Fasting FE<sub>Na</sub>, %                 | 0.03 ± 0.14                  | -0.15 ± 0.14                  | .062                                             | -0.25 ± 0.18               | -0.37 ± 0.24            | .490                                             | -0.04 (-0.48 to 0.41)                          | .869 |
| Postprandial FE<sub>Na</sub>, %                | 10.79 ± 1.52                 | 11.51 ± 1.26                  | .382                                             | 13.25 ± 1.10               | 13.47 ± 0.93            | .677                                             | 0.01 (-1.92 to 1.93)                           | .995 |
| Postprandial FE<sub>Urea</sub>, %             | 70.78 ± 3.23                 | 68.15 ± 3.84                  | .081                                             | 66.83 ± 2.04               | 68.45 ± 2.56            | .548                                             | -4.9 (-12.71 to 2.82)                          | .199 |
| Postprandial urinary pH                       | 5.56 ± 0.19                  | 5.47 ± 0.11                   | .477                                             | 5.25 ± 0.10                | 5.42 ± 0.12             | .193                                             | -0.10 (-0.42 to 0.22)                          | .511 |
| Postprandial urine osmolality, mOsm/kg         | 219 [165-368]                | 219 [165-368]                 | .317                                             | 237 [164-324]              | 192 [160-290]           | .593                                             | 24 (-26 to 75)                                 | .326 |

Note: 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 with glimepiride. Paired t-tests or Wilcoxon signed rank tests were used for within-group comparisons. Significant differences are indicated by bold font.

Abbreviations: ERPF, effective renal plasma flow; FE<sub>Na</sub>, fractional potassium excretion; FE<sub>Na</sub>, fractional sodium excretion; FE<sub>Urea</sub>, fractional urea excretion; FF, filtration fraction; GFR, glomerular filtration rate; P<sub>GLO</sub>, glomerular hydraulic pressure; PP, postprandial; R<sub>A</sub>, afferent renal arteriolar resistance; RBF, renal blood flow; R<sub>E</sub>, efferent renal arteriolar resistance; RVR, renal vascular resistance.
postprandial PRC, TAM insulin, or TAM glucagon from baseline to week 8. From baseline to week 8, linagliptin increased TAM intact GLP-1 by $3.7 \pm 0.9$ pmol/L ($P = .005$), which was significantly more than the increase induced by glimepiride ($+2.72$ pmol/L [0.38 to 5.06; $P = .025$]; Appendix S6, Figure 1).

3.5 Exploratory correlation analyses

Correlation analyses between changes from baseline to week 8 in postprandial FF and selected factors associated with renal haemodynamics are presented in Appendix S11. In the linagliptin group, change in postprandial FF correlated positively with change in postprandial MAP ($R = 0.807; P = .009$) and TAM glucagon ($R = 0.782; P = .008$; Appendix S12), but not with changes in TAM glucose, TAM insulin, TAM intact GLP-1, postprandial PRC, or postprandial FENa (Appendix S11). Change in postprandial glucagon was associated with change in $R_G$ ($R = 0.830; P = .003$; Appendix S13), but not with MAP, FENa, or $F_E$.

4 DISCUSSION

The mechanisms underlying the glucose-independent benefits of DPP-4is on albuminuria onset and progression in people with T2D and high cardiorenal risk in several phase III and cardiovascular outcome trials remain incompletely understood. We hypothesized that a DPP-4i reduces postprandial hyperfiltration, an emerging renal risk factor in T2D. To the best of our knowledge, this is the first study to assess the effect of a DPP-4i or sulphonylurea on postprandial renal haemodynamics. Eight-week treatment with linagliptin compared with glimepiride did not reduce postprandial hyperfiltration after a liquid meal in overweight adults with T2D with normal renal function. Indeed, and contrary to our hypothesis, linagliptin increased FF by 2.1 percentage points and estimated $P_{GLO}$ by 3.0 mmHg, possibly by increasing estimated $R_G$. The clinical significance of this finding merits further study. As both treatments reduced postprandial plasma glucose excursions to a similar extent, and correction for HbA1c difference did not alter results, the modest linagliptin-induced postprandial hyperfiltration may be considered a glucose-independent and thereby drug-specific effect. Exploratory correlation analyses suggest that postprandial blood pressure and possibly circulating glucagon, rather than other measured vasoactive hormones, directly or indirectly mediate the suggested meal-induced hyperfiltration following linagliptin therapy. Linagliptin compared with glimepiride did not significantly affect postprandial tubular functions.

Accumulating evidence suggests a prognostic and pathogenic role of glomerular hyperfiltration in diabetic kidney disease initiation and progression. The relevance of targeting hyperfiltration as a renal risk factor is illustrated by recent clinical trials involving various medicinal products, including sodium-glucose co-transporter-2 inhibitors. The effect of DPP-4is on renal haemodynamics was extensively studied in the fasting state, showing no clinically meaningful properties of sitagliptin or linagliptin versus placebo, or linagliptin versus glimepiride, on measured and estimated indices of glomerular hyperfiltration in people with T2D. Mechanistic studies that assess (pleiotropic) drug effects in humans are classically performed under fasting conditions, while most humans spend most of their day in a fed state. Yet the physiological meal-induced increase in glomerular pressure and filtration—in the setting of an intact renal functional reserve—is believed to add to the overall renal risk in people with T2D. As such, insight into the integrated pleiotropic effects of a drug (e.g. in a...
composite risk score that incorporates all favourable/unfavourable drug effects on risk factors, such as the PRE-score\textsuperscript{22} on cardiorenal risk over 24 hours, including both the fasting and fed state, is not possible from mechanistic trials. As DPP-4is exert their most pronounced drug effects postprandially, by blocking the degradation of incretin hormones, we hypothesized that this drug class may particularly beneficially influence meal-induced renal haemodynamics. Unexpectedly, compared with glimepiride, linagliptin therapy induced glomerular hyperfiltration, for which the clinical significance remains speculative. These results suggest that the renoprotective potential of DPP-4is is modestly constrained by drug-induced postprandial hyperfiltration in people with T2D with intact renal functional reserve (i.e. those capable of exploiting their entire filtration capacity upon an external ‘stressor’, such as a protein-rich meal, or infusion of amino acids or dopamine\textsuperscript{1,22}). Moreover, as GLP-1RAs do not affect postprandial hyperfiltration,\textsuperscript{16} our finding may partly elucidate why DPP-4is appear to exhibit fewer benefits on renal endpoints versus GLP-1RAs,\textsuperscript{24} although many other relevant differences between the two incretin-based drug classes may also play a role.\textsuperscript{25} In turn, our observation may also explain the reported potential of DPP-4is to reduce albuminuria in people with T2D with more advanced chronic kidney disease,\textsuperscript{3} who in the fasting state already exploit their full filtration capacity to compensate for a reduction in nephron mass.\textsuperscript{1}

While DDP-4is may predispose to nephron damage in the postprandial state, in parallel they may aid the gut-renal axis to maintain water/solute-balance. In the current study, compared with glimepiride, linagliptin reduced change in postprandial plasma potassium and urea without affecting tubular functions. This requires further study, particularly in patients in whom electrolyte/solute-homeostasis is of high clinical importance.

In exploratory correlational analyses, we attempted to assess the mechanisms underlying the observed DPP-4i–induced postprandial hyperfiltration. Foremost, we observed a positive association between change from baseline in postprandial FF and MAP. In a secondary analysis of our trial, linagliptin blunted the meal-induced decrease in circulating levels of other non-GLP-1 peptides, such as SDF-1a, neuropeptide-Y, substance-P, and many others (at least >40).\textsuperscript{26} These DPP-4 substrates beyond GLP-1 may also decrease renal autoregulation efficiency. Second, the positive correlation between change in postprandial FF and postprandial glucagon in the linagliptin group is of interest. Because glucagon secretion increases after ingestion of a protein-rich meal, and glucagon infusion may increase GFR,\textsuperscript{2} its role in postprandial hyperfiltration in the gut-renal axis is suspected. In the current trial, an increase in postprandial glucagon was associated with increased R\textsubscript{a}, suggesting direct vasoconstrictive actions of this hormone on renal efferent arterioles. However, because a number of studies did not identify specific glucagon receptors in glomeruli, or afferent or efferent arterioles,\textsuperscript{2} glucagon is not probable to directly act on the glomerulus. In a series of elegant micropuncture studies in normal rats, a high-protein diet and glucagon infusion reduced the concentration of NaCl in the early distal tubule, leading to a diminished signal for TGF at the macula densa, thereby diminishing the TGF-dependent restraint of GFR (mostly via R\textsubscript{a}, and in some cases via R\textsubscript{c}), thus permitting GFR to rise.\textsuperscript{2} We expected that linagliptin would decrease postprandial glucagon concentrations as previously reported,\textsuperscript{27} and—together with GLP-1–induced proximal natriuresis by inhibition of NHE-3 activity—would reduce postprandial hyperfiltration via TGF. Based on current findings, it appears that those adults with T2D with a (paradoxical) increase or least postprandial decrease in circulating glucagon, experience the most pronounced renal consequences of impaired autoregulation and increases in arterial pressure. Yet we did not observe an effect of DPP-4is on postprandial R\textsubscript{a} or FE\textsubscript{Na}, or find a correlation between these indices and FF, suggesting that TGF perhaps remains irrelevantly affected. Taken together, the partly unexplained correlation between glucagon and FF in the linagliptin group should be interpreted with caution in this comparatively small-sized study, and should serve as hypothesis generating for future mechanistic studies. Finally, as GLP-1/Glucagon receptor administration reduces PRC and angiotensin-II concentration in some studies,\textsuperscript{10,28–30} we hypothesized that DPP-4i treatment could ameliorate RAS-mediated hyperfiltration. However, linagliptin did not affect PRC, nor was there an association between change in PRC and FF or R\textsubscript{c}. Of note, a potential reduction in R\textsubscript{c} through angiotensin-II lowering may have been blunted by the extensive use of RAS inhibition in the current trial. Collectively, we hypothesize that DPP-4is may exacerbate the activity of physiological factors involved in the gut-renal axis, leading to increased glomerular pressure and filtration. However, as membrane-bound DPP-4 has been located at various sites in the kidney (e.g. preglomerular vascular smooth muscle cells, mesangial cells, podocytes, and proximal tubule), we cannot rule out direct effects of the drug on renal physiology.

Our study has some limitations. First, the sample size of our investigational substudy is comparatively small, potentially leading to heterogeneity. We attempted to minimize the effect of a small sample size by using homogeneous study groups and by conducting a pretest preparation phase with a particular emphasis on standardization of factors that could potentially influence neurohormonal activation, such as dietary sodium and protein intake. However, by including a predominantly elderly patient population (~65 years of age), our results cannot be generalized to a younger population with T2D, and small imbalances in co-morbidities (e.g. cardiorenal risk) and co-medication (e.g. RAS inhibitors) may have influenced our results and
clinical interpretation. In line with this, we are unable to rule out gender differences in the renal responses to the drug treatments, as an imbalance in the male/female ratio was present at baseline (four [31%] vs. no male patients randomized to glimepiride and linagliptin, respectively). In sensitivity analyses, we do note that the outcomes were unchanged when only male patients were taken into account. Second, as profound urine collection errors based on inulin-excretion analyses were present in the fasting state—requiring us to use the continuous infusion method for fasting renal haemodynamics in these patients15—we are not able to construct and assess the renal functional reserve at baseline (i.e. the change in GFR from fasting to the postprandial state), nor evaluate the meal-induced effects of the study drugs on GFR, ERPF, and FF per se. Also, given these constraints, we are unable to assess between-group differences in renal functional reserve at baseline. Third, the significant reduction in HbA1c with glimepiride versus linagliptin relative to baseline may have influenced the outcome (e.g. via structural changes in the kidney). However, at the time of postprandial renal assessment, TAM glucose excursion was not significantly different between groups at baseline or long-term follow-up, and correction for HbA1c differences did not fundamentally influence the main results. Finally, the Gomez equations necessitate assumptions,18 which are insufficiently validated in the postprandial state.

In conclusion, 8 weeks of treatment with linagliptin compared with glimepiride did not improve postprandial haemodynamics, and did not affect tubular functions, when added to metformin in overweight T2D patients without renal impairment. Linagliptin did not lead to a presumed postprandial TGF activation and inhibition of RAS activity, which was part of our predefined hypothesis. Indeed, and contrary to our hypothesis, the data suggest that linagliptin modestly induced postprandial hyperfiltration, of which the clinical relevance remains speculative. Yet this observation suggests a factor that constrains its kidney-protective potential in patients with T2D. In general, we call for mechanistic studies that assess the drug-induced effects on postprandial renal haemodynamic and tubular functions, to gain insight into the integrated 24-hour pleiotropic effects of pharmacological agents.

ACKNOWLEDGEMENTS
In memory of Prof. Michaela Diamant, whose experience and expertise were crucial for the design of this study. The authors extend their gratitude to all the study participants who took part in this research study for their time and commitment to the demanding study protocol. Furthermore, we thank the study nurses for their excellent practical support during the conduct of this study, with special thanks to Sandra Gassman and Jeannette Borop (Diabetes Center, Department of Internal Medicine, Amsterdam University Medical Centers, location VUMC, Amsterdam, The Netherlands). Finally, we thank Adele Dijk and Nel Willekes-Koolschijn (Department of Nephrology and Hypertension, University Medical Center, Utrecht, The Netherlands) for their much appreciated technical laboratory assistance. Parts of these data were presented at the European Diabetic Nephropathy Study Group Meeting (Helsinki, Finland, 19-20 May 2017). This was an investigator-initiated study, entirely planned and conducted under the scientific supervision of Prof. Michaela Diamant and, after her passing in April 2014, M.H.H.K. and D.H.v.R. Funding for the study was provided by Boehringer Ingelheim. The funder had no role in the study design, the analyses or interpretation of the data, or drafting of the manuscript. The funder had no role in the decision to submit this manuscript for publication.

CONFLICT OF INTEREST
M.H.A.M. is a speaker/consultant for AstraZeneca, Eli Lilly & Co., Novo Nordisk, and Sanofi. L.T. consulted for Eli Lilly & Co. and Novo Nordisk. Through M.H.H.K., the Amsterdam University Medical Centers, location VUMC, received research grants from Boehringer Ingelheim, Novo Nordisk, and Sanofi. D.H.v.R. serves on advisory boards of Boehringer Ingelheim, Eli Lilly Alliance, Novo Nordisk, Sanofi, and Merck Sharp & Dohme (MSD), and received research grants from AstraZeneca, Boehringer Ingelheim, Eli Lilly, Sanofi, and MSD. J.J.H. has been a member of advisory boards for Novo Nordisk. No other potential conflicts of interest relevant to this article were reported.

AUTHOR CONTRIBUTIONS
M.H.A.M. participated in the design and planning of the study, coordinated the test visits and performed measurements, performed statistical analyses, produced the graphical representation of the data, interpreted the data, and wrote the manuscript. L.T. helped with data collection, interpreted the data, and critically reviewed the manuscript. M.M.S., M.H.H.K., J.A.J., and D.H.v.R. contributed to the interpretation of the data, discussion of the intellectual content, and critical review of the manuscript. D.M.O., B.H., J.J.H., and A.H.J.D. generated data and/or contributed to the discussion of the intellectual content and critical review of the manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication. M.H.A.M. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

PEER REVIEW
The peer review history for this article is available at https://publons.com/publon/10.1111/dom.14557.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID
Marcel H. A. Muskiet https://orcid.org/0000-0003-4116-555X
Lennart Tonneijck https://orcid.org/0000-0001-5637-910X
Mark M. Smits https://orcid.org/0000-0001-8236-8842

REFERENCES
1. Tonneijck L, Muskiet MH, Smits MM, et al. Glomerular hyperfiltration in diabetes: mechanisms, clinical significance, and treatment. J Am Soc Nephrol. 2017;28(4):1023-1039.
16. Tonneijck L, Muskiet MHA, Smits MM, et al. Postprandial renal
function: from physiology to pharmacology and outcomes in diabetes. Nat Rev Nephrol. 2017;13(10):605-628.

14. Lovshin JA, Rajasekeran H, Lytvyn Y, et al. Dipeptidyl peptidase 4 inhibi-
tor linagliptin versus sulphonylurea glimepiride in systemic haemodynamics in overweight patients with type 2 diabetes: a secondary analysis of an 8-week, randomized, controlled, double-blind trial. Diabetes Obes Metab. 2020;22:1847-1856.

12. Tonneijck L, Muskiet MHA, Wheeler DC, Heerspink HJL. New pharmacological strategies for protecting kidney function in type 2 diabetes. Lancet Diabetes Endocrinol. 2019;7(5):397-412.

10. Kraaijenhof J, Muskiet MHA, Tonneijck L, et al. Effects of dipeptidyl peptidase-4 inhibitor linagliptin versus sulphonylurea glimepiride in overweight patients with type 2 diabetes: a predefined substudy of a randomized, double-blind trial. Diabetes Obes Metab. 2020;22:115-124. doi: 10.1111/dom.14557

SUPPORTING INFORMATION
Additional supporting information may be found in the online version of the article at the publisher’s website.

How to cite this article: Muskiet MHA, Tonneijck L, Smits MM, et al. Postprandial renal haemodynamic effects of the dipeptidyl peptidase-4 inhibitor linagliptin versus the sulphonylurea glimepiride in adults with type 2 diabetes (RENASIL): A predefined substudy of a randomized, double-blind trial. Diabetes Obes Metab. 2020;22(1):115-124. doi: 10.1111/dom.14557

MUSKIJET ET AL.

2. Bankir L, Rousset R, Bouby N. Protein- and diabetes-induced glomerular hyperfiltration: role of glucagon, vasopressin, and urea. Am J Physiol Renal Physiol. 2015;309(1):F2-F23.

3. Muskiet MHA, Tonneijck L, Smits MM, et al. GLP-1 and the kidney: from physiology to pharmacology and outcomes in diabetes. Am J Physiol Renal Physiol. 2015;308(8):F867-F877.

5. Perkovic V, Toto R, Cooper ME, et al. Effects of linagliptin on cardio-
vascular and kidney outcomes in patients with normal and reduced kid-
ney function: secondary analysis of the CARMELINA randomized trial. Diabetes Care. 2020;43(8):1803-1812.

6. Cornel JH, Bakris GL, Stevens SR, et al. Effect of sitagliptin on kidney function and respective cardiovascular outcomes in type 2 diabetes: outcomes from TECOS. Diabetes Care. 2016;39(12):2304-2310.

Jensen EP, Poulsen SS, Kissow H, et al. Activation of GLP-1 receptors on vascular smooth muscle cells reduces the autoregulatory response in afferent arterioles and increases renal blood flow. Am J Physiol Renal Physiol. 2015;308(8):F867-F877.

8. Muskiet MH, Tonneijck L, Smits MM, et al. Acute renal haemodynamic effects of glucagon-like peptide-1 receptor agonist exenatide in healthy overweight men. Diabetes Obes Metab. 2016;18(2):178-185.

11. Tonneijck L, Smits MM, Muskiet MH, et al. Acute renal effects of the dipeptidyl peptidase-4 inhibitor linagliptin versus sulphonylurea glimepiride on systemic haemodynamics in overweight patients with type 2 diabetes: a secondary analysis of an 8-week, randomized, controlled, double-blind trial. Diabetes Obes Metab. 2020;22:1847-1856.

13. Ott C, Kistner I, Keller M, et al. Effects of linagliptin on renal endothe-
lar hyperfiltration: role of glucagon, vasopressin, and urea. Am J Physiol Renal Physiol. 2015;309(1):1143-1155.

14. Lovejoy C, Kistner I, Keller M, et al. Effects of linagliptin on renal endothe-
lar hyperfiltration: role of glucagon, vasopressin, and urea. Am J Physiol Renal Physiol. 2015;308(8):F867-F877.

15. Tonneijck L, Smits MM, Muskiet MH, et al. Effects of DPP-4 inhibi-
tor linagliptin versus sulfonylurea glimepiride as add-on to metformin on renal physiology in overweight patients with type 2 diabetes (RENASIL): a randomized, double-blind trial. Diabetes Care. 2020;43(11):2889-2893.

16. Tonneijck L, Muskiet MHA, Smits MM, et al. Postprandial renal haemodynamic effect of lixisenatide vs once-daily insulin-glimepiride in patients with type 2 diabetes on insulin-glimepiride: an 8-week, randomised, open-label trial. Diabetes Obes Metab. 2017;19(12):1669-1680.

17. Gomez DM. Evaluation of renal resistances, with special reference to changes in essential hypertension. J Clin Invest. 1951;30(10):1143-1155.

18. Bjornstad P, Skrtic M, Lytvyn Y, Maaks DM, Johnson RJ, Cherney DZ. The Gomez’ equations and renal functional hemodynamics in kidney disease research. Am J Physiol Renal Physiol. 2016;311(5):F967-F975.

19. Mosteller RD. Simplified calculation of body-surface area. N Engl J Med. 1987;317(10):1098.

20. Kraaijenhof J, Muskiet MHA, Tonneijck L, et al. Effects of dipeptidyl peptidase-4 inhibitor linagliptin versus sulphonylurea glimepiride on systemic haemodynamics in overweight patients with type 2 diabetes: a secondary analysis of an 8-week, randomized, controlled, double-blind trial. Diabetes Obes Metab. 2020;22:1847-1856.

21. Muskiet MHA, Wheeler DC, Heerspink HJL. New pharmacological strategies for protecting kidney function in type 2 diabetes. Lancet Diabetes Endocrinol. 2019;7(5):397-412.

22. Heerspink HJ, Grobbee DE, de Zeeuw D. A novel approach for esta-
blishing cardiovascular drug efficacy. Nat Rev Drug Discov. 2014;13(12):942.

23. van Londen M, Schaeffers A, de Borst MH, Joles JA, Navis G, Lely AT. Overweight young female kidney donors have low renal functional reserve postdonation. Am J Physiol Renal Physiol. 2018;315(3):F454-F459.

24. MANN JFE, Muskiet MHA. Incretin-based drugs and the kidney in type 2 diabetes: choosing between DPP-4 inhibitors and GLP-1 receptor agonists. Kidney Int. 2021;99(2):314-318.

25. Nauck M. Incretin therapies: highlighting common features and differ-
ces in the modes of action of glucagon-like peptide-1 receptor ago-
nists and dipeptidyl peptide-4 inhibitors. Diabetes Obes Metab. 2016;18(3):203-216.

26. Muskiet MH, Smits MM, Monsink LM, Diamant M. The gut-renal axis: do incretin-based agents confer renoprotection in diabetes? Nat Rev Nephrol. 2014;10(2):88-103.

27. Ahren B, Landin-Olsson M, Jansson PA, Svensson M, Holmes D, Schweizer A. Inhibition of dipeptidyl peptide-4 reduces glycaemia, sustains insulin levels, and reduces glucagon levels in type 2 diabetes. J Clin Endocrinol Metab. 2004;89(5):2078-2084.

28. Skov J, Dejgaard A, Frokiaer J, et al. Glucagon-like peptide-1 (GLP-1): effect on kidney hemodynamics and renin-angiotensin-aldosterone system in healthy men. J Clin Endocrinol Metab. 2013;98(4):E664 - E671.

29. Asmar A, Simonsen L, Asmar M, et al. Renal extraction and acute effects of glucagon-like peptide-1 on central and renal hemodynamics in healthy men. Am J Physiol Endocrinol Metab. 2015;308(8):E641 - E649.

30. Tonneijck L, Muskiet MHA, Smits MM, van Raalte DH, Diamant M. Combining incretin-based drugs and RAAS inhibitors: more cons than pros? Lancet Diabetes Endocrinol. 2014;2(9):684-685.