Dipeptidyl Peptidase 4 Inhibition Stimulates Distal Tubular Natriuresis and Increases in Circulating SDF-1α^{1-67} in Patients With Type 2 Diabetes

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OBJECTIVE

Antihyperglycemic agents, such as empagliflozin, stimulate proximal tubular natriuresis and improve cardiovascular and renal outcomes in patients with type 2 diabetes. Because dipeptidyl peptidase 4 (DPP-4) inhibitors are used in combination with sodium–glucose cotransporter 2 (SGLT2) inhibitors, we examined whether and how sitagliptin modulates fractional sodium excretion and renal and systemic hemodynamic function.

RESEARCH DESIGN AND METHODS

We studied 32 patients with type 2 diabetes in a prospective, double-blind, randomized, placebo-controlled trial. Measurements of renal tubular function and renal and systemic hemodynamics were obtained at baseline, then hourly after one dose of sitagliptin or placebo, and repeated at 1 month. Fractional excretion of sodium and lithium and renal hemodynamic function were measured during clamped euglycemia. Systemic hemodynamics were measured using noninvasive cardiac output monitoring, and plasma levels of intact versus cleaved stromal cell–derived factor (SDF)-1α were quantified using immunoaffinity and tandem mass spectrometry.

RESULTS

Sitagliptin did not change fractional lithium excretion but significantly increased total fractional sodium excretion (1.32 ± 0.5 to 1.80 ± 0.01% vs. 2.15 ± 0.6 vs. 2.02 ± 1.0%, P = 0.012) compared with placebo after 1 month of treatment. Moreover, sitagliptin robustly increased intact plasma SDF-1α^{1-67} and decreased truncated plasma SDF-1α^{3-67}. Renal hemodynamic function, systemic blood pressure, cardiac output, stroke volume, and total peripheral resistance were not adversely affected by sitagliptin.

CONCLUSIONS

DPP-4 inhibition promotes a distal tubular natriuresis in conjunction with increased levels of intact SDF-1α^{1-67}. Because of the distal location of the natriuretic effect, DPP-4 inhibition does not affect tubuloglomerular feedback or impair renal hemodynamic function, findings relevant to using DPP-4 inhibitors for treating type 2 diabetes.

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Cardiovascular and renal complications are leading causes of premature death and morbidity in patients with type 2 diabetes. Diabetic nephropathy is independently associated with an increased risk for adverse cardiovascular outcomes and is partly driven by ambient hyperglycemia (1). Unfortunately, preventing renal dysfunction or slowing progression of chronic kidney and/or cardiovascular disease in type 2 diabetes remains a major therapeutic shortcoming of current diabetes clinical care.

Dipeptidyl peptidase 4 (DPP-4) inhibitors are antihyperglycemic agents that potentiate incretin action and are widely used in the treatment of type 2 diabetes (2) as monotherapy or, more commonly, in combination with metformin, sulfonylureas, and sodium–glucose cotransporter 2 (SGLT2) inhibitors. DPP-4 inhibitors lower glucose by preventing the N-terminal cleavage and inactivation of the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (3,4). The cardiovascular safety of DPP-4 inhibition with sitagliptin (5), alogliptin (6), and saxagliptin (7) has been demonstrated in large outcome studies; however, saxagliptin and alogliptin may increase hospitalization for heart failure, particularly in patients with preexisting heart or kidney disease (8). Remarkably, little is known about the renal hemodynamic or natriuretic actions of DPP-4 inhibitors in subjects with type 2 diabetes.

The results of the BI 10773 (Empagliflozin) Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients (EMPA-REG OUTCOME) trial (9) with the SGLT2 inhibitor, empagliflozin, highlight the clinical importance of understanding how different glucose-lowering agents modulate natriuresis and renal hemodynamic function. Although sitagliptin increased fractional sodium excretion in a single study of subjects with type 2 diabetes (10), the mechanism(s) and tubular location for these effects and associated changes in renal hemodynamic or cardiovascular function have not been comprehensively elucidated. Remarkably, cardioprotection with empagliflozin was evident within weeks, highlighting the importance of understanding the acute nonglycemic actions of current therapies used to treat type 2 diabetes.

Accordingly, we have now studied whether and how the DPP-4 inhibitor sitagliptin modifies proximal and distal renal tubular sodium handling and also renal and systemic hemodynamic function after acute and sustained administration. Here we demonstrate that sitagliptin enhances natriuresis independently of significant changes in renal or systemic hemodynamic parameters, likely through sites and mechanisms distinct from those regulated by SGLT2 inhibitors in the kidney. Furthermore, we demonstrate that sitagliptin robustly augments plasma levels of bioactive stromal cell–derived factor (SDF)-$1\alpha^{1\text{-}67}$, a chemokine with proven natriuretic actions in preclinical studies. Collectively, our findings highlight distinct mechanisms for how DPP-4 inhibitors (vs. effects previously elucidated for SGLT2 inhibitors) control natriuresis and tubuloglomerular function, results with implications for the use of these drugs as combination therapy in subjects with type 2 diabetes.

RESEARCH DESIGN AND METHODS

Study Population

Local advertising was used to enroll 36 patients with type 2 diabetes (A1C 6.5–9.0% [48–75 mmol/L], or HbA_{1c} <6.0–9.0% [742–75 mmol/mol] if treated with oral hypoglycemic agents or insulin and diagnosed with type 2 diabetes >2 years' duration), receiving stable doses (within 1 month) of renin-angiotensin-aldosterone system (RAAS) blockade (ACE inhibitor or angiotensin receptor blocker if ACE inhibitor intolerant), with a systolic blood pressure (SBP) of at least >120 mmHg and estimated glomerular filtration rate (GFR) >50 mL/min/1.73 m^2 (Supplementary Fig. 1; see Supplementary Table 1 for exclusion criteria). The study was approved by the University Health Network Research Ethics Board and conducted in accordance with the Declaration of Helsinki and International Conference on Harmonization on Good Clinical Practice. All patients provided written informed consent.

Study Design

This was a single-center (University Health Network, University of Toronto, Toronto, Ontario, Canada), prospective, double-blind, randomized, placebo-controlled trial, conducted over two study visits separated by 1 month (Supplementary Fig. 2). Thirty-six patients were randomized to sitagliptin (100 mg per os daily) or to identical placebo per os daily (manufactured and supplied by Merck Sharpe & Dohme) by the research trial pharmacist with an allocation of one to one and a block size of four using computer-generated lists (Supplementary Fig. 1).

Measurement of Renal Tubular Function and Hemodynamics

One week before the first study visit (study visit 1), study participants were instructed to maintain a minimum sodium intake of 150 mmol/day and a protein diet of 1.5 g/kg/day and to avoid alcohol and tobacco for at least 48 h. In the 24 h leading up to study visit 1, study participants completed a 24-h timed urine collection. The night before study visit 1, study participants fasted for a minimum of 12 h and avoided caffeine intake. Participants were also instructed to take one lithium carbonate tablet (300 mg) at 2200 h to allow for measurement of fractional lithium excretion (FE_{Li}). FE_{Li} is a marker of proximal tubular sodium reabsorption, which allows for determination of proximal versus distal natriuresis localization within the renal tubules. On the morning of study visit 1, study participants withheld all oral antihyperglycemic agents and prandial insulin but continued all other routine medications, including antihypertensives.

Study participants arrived at the Renal Physiology Laboratory (Toronto General Hospital, Toronto, Ontario, Canada) at ~0800 h for a brief physical examination and body weight measurement. After a rest period of ~15 min, blood work (HbA_{1c}, blood glucose, electrolytes, lipids, creatinine, urea, uric acid, aldosterone, plasma renin concentration) and urine parameters (albumin, protein, electrolytes) were collected, and vital signs were measured at the bedside. A standardized liquid meal (2 MightyShakes Vanilla 50/6 fl oz [single serving, 240 calories: fat, 13 g; carbohydrate, 23 g; protein, 8 g]; Hormel Health Laboratories, Savannah, GA) was given to all study participants to allow for endogenous incretin hormone secretion and to control dietary intake during the study visits. For participants who could not tolerate the standardized liquid meal (three patients), a substitute meal with equal proportions of macronutrients was given (ad libitum water consumption was allowed during the experimental period, up to a maximum of 500 mL).

A euglycemic clamp was initiated, and once euglycemia was reached and

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stabilized for 2 h, a third venous catheter was placed to facilitate bolus infusion of *p*-aminohippurate sodium 20% (PAH; Bachem, Bubendorf, Switzerland) and inulin (Inutest; Fresenius Kabi Austria GmbH, Graz, Austria) to assess effective renal plasma flow (ERPF) and GFR, respectively, according to previously described methods (11). After a 90-min equilibration period, urine was collected by spontaneous voiding, and blood was sampled every 45 min.

After baseline renal function tests were completed at ~1300 h, study participants were given an identical fixed-calorie liquid meal (as described above) to stimulate endogenous incretin hormone secretion. Participants were then given their first dose of study drug (sitagliptin or placebo). Participants were asked to empty their bladders (they were upright and asked to void until a subjective feeling of empty was achieved), and urine was collected under spontaneous voiding for up to 3 h during a timed urine collection. PAH and inulin were continuously infused at maintenance levels through this 3-h period, and blood samples were drawn hourly for measurement of GFR_{INULIN} and $ERPF_{PAH}$ in response to the study drug.

After study visit 1, subjects were instructed to continue all routine medications in addition to the daily study drug. An interim telephone call was made to study participants at ~14 days to screen for adverse events (study visit 2). Approximately 28–30 days later (1 month), all study participants returned to the Renal Physiology Laboratory for a final study visit, study visit 3. All prestudy procedures (e.g., dietary sodium and protein recommendations) and experimental procedures on study visit 3 were identical to study visit 1, with the exception that on the morning of study visit 3, participants withheld study drug administration until given by study personnel after the second standardized liquid meal (~1300 h) before the timed urine collection during study visit 3.

Noninvasive Cardiac Output Monitoring

Cardiovascular parameters were assessed to detect potential systemic physiological effects that might arise secondary to DPP-4 inhibitor–stimulated natriuresis. Systemic hemodynamic function was measured using electrical bioreactance-based noninvasive cardiac output monitoring methods (NICOM; Cheetah Medical, Newton Center, MA). In brief, four electrodes were placed externally on the chest (at the clavicle) and lower abdomen (below costal margin) bilaterally. Calibration and baseline measurements were completed before study drug administration, and then measurements were done hourly (first 10 min) for 3 h after study drug administration. Patients were positioned supine during measurements and were instructed to remain still and quiet.

Study End Points

The primary study end point was the change in the fraction of excreted sodium (FE_{Na}) at 3 h after a single dose and after short-term administration of sitagliptin (1 month). Secondary end points included the change in gold standard measured renal hemodynamic function, GFR_{INULIN} and $ERPF_{PAH}$ (12), under clamped euglycemia, and derived measures of intrarenal hemodynamics (renal blood flow, filtration fraction [FF], renal vascular resistance, glomerular hydrostatic pressure, renal afferent resistance, renal efferent resistance), FE_{Li}, systemic hemodynamic function (cardiac output, stroke volume, total peripheral resistance, mean arterial pressure, SBP, diastolic blood pressure, heart rate, and pulse pressure) using previously described methods (13,14). FE_{Na} and FE_{Li} excretion were calculated according to FE(electrolyte) = 100 × ([(electrolyte urine) × (creatinine plasma)]/[(electrolyte plasma) × (creatinine urine)]).

Acute and chronic changes in plasma SDF-1$\alpha^{1\text{-}67}$, urinary inflammatory cytokines and chemokines, plasma renin concentration, aldosterone, plasma and urinary nitric oxide (NO), plasma atrial natriuretic peptide (ANP), and urinary reactive oxygen species (8-isoprostane, 8-hydroxy-2-deoxyguanosine, cyclic guanosine monophosphate [cGMP]) were secondary exploratory end points. Tertiary confirmatory end points consisted of changes in HbA_{1c}, lipids (total cholesterol, LDL, HDL, triglycerides), and body weight.

Biochemical Analyses

Routine blood (HbA_{1c}, electrolytes, liver enzymes, and urine biochemistry) were performed at baseline and after 1 month of treatment using conventional assay methods by the Department of Clinical Biochemistry at the University Health Network, Toronto, Ontario, Canada. Urine lithium was measured by inductively coupled plasma spectroscopy by n-Common Laboratories, North York, Ontario, Canada.

Urinary inflammatory cytokines, chemokines, and growth factors and cognate receptors were measured using a Discovery Assay (Human Cytokine Array/Chemokine Array 42-Plex; Eve Technologies Corp., Calgary, Alberta, Canada), using previously described methods, corrected for urinary creatinine (15). Plasma ANP, plasma and urinary NO, plasma and urinary cGMP, urine 8-isoprostane, and urine 8-hydroxy-2-deoxyguanosine were measured by ELISA, as described previously (16,17).

Plasma SDF-1α and Sitagliptin Quantification

Levels of intact and truncated SDF-1α in plasma were measured using a hybrid method coupling immunoaffinity enrichment and liquid chromatography–tandem mass spectrometry (18). Briefly, plasma SDF-1$\alpha^{1\text{-}67}$ and SDF-1$\alpha^{3\text{-}67}$ were first captured with anti–SDF-1α antibody (MAB350; Bio-Techne, R&D Systems, Inc., Minneapolis, MN), immobilized on magnetic beads, and then quantified using a multiple reaction monitoring method on a triple quadrupole mass spectrometer. Plasma sitagliptin concentrations were quantified as described previously (19).

Statistical Analyses

Statistical analyses were performed by an independent statistician using SAS 9.2 (SAS Institute Inc.). Data are reported as mean ± SD, median (interquartile range), or as frequency (%). The treatment effect on all study end points was assessed using repeated-measures linear mixed-effects models. The sample size calculation was based on the estimated effect of study medication on $FE_{Na.}$ Preclinical studies indicated the most conservative estimate for a DPP-4 inhibitor on FE_{Na} is 14% (20). No variances were provided for the preclinical studies, so we used an "inflated" SD of 14 (equal to the effect size) in our sample size calculation. With this variance and effect size, a sample size of 16 per group provided >80% power to detect significant between-group differences (two-tailed α = 0.05) in response

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to DPP-4 inhibition. Unblinding occurred after completion of the study and locking of the database, and analyses were done on a per-protocol basis. One participant was excluded from analysis of NICOM measurements due to an inability to obtain reliable NICOM measurements because of large body habitus (allocated to placebo), and one participant's 3-h NICOM measurements were discarded due to the inability to capture data (allocated to placebo). An α level of 0.05 was used to determine statistical significance for all comparisons.

RESULTS

Clinical Characteristics

In total, 48 patients were screened between July 2015 and March 2016 at the Renal Physiology Laboratory, Toronto General Hospital, Toronto, Ontario, Canada (Supplementary Fig. 2), and 36 patients were ultimately randomized to sitagliptin (n = 18) or placebo (n = 18). After randomization but before study visit 1, one patient (allocated to sitagliptin) withdrew from the study. Three patients were withdrawn during the study because of adverse events (dyspnea, allocated to placebo; vasovagal event, allocated to placebo; lower gastrointestinal bleeding, allocated to sitagliptin). Baseline characteristics were similar between both groups (Table 1). Intravenous infusion volumes of dextrose (5%), insulin, and maintenance fluids did not differ significantly between treatment groups (data not shown).

Table 1—Demographics and clinical characteristics of study population

	Placebo (n = 16)		Sitagliptin (n = 16)	
	Day 1	1 Month	Day 1	1 Month
Demographics				
Age (years)	59.3 ± 8.8	—	60.4 ± 7.6	—
Males	9 (56.3)	—	11 (68.8)	—
Ethnicity				
Caucasian	8 (50)	—	12 (75)	—
South Asian	4 (25)	—	2 (12.5)	—
Black	1 (6.3)	—	1 (6.3)	—
Other	3 (18.7)	—	2 (6.2)	—
Diabetes duration (years)	8.50 (5.5, 14.0)	—	6.0 (3.0, 10.0)	—
Metformin use	14 (87.5)	14 (87.5)	12 (75)	12 (75)
Sulfonylurea use	6 (37.5)	6 (37.5)	3 (18.8)	3 (18.8)
Insulin use	4 (25)	4 (25)	4 (25)	4 (25)
Hypertension duration (years)	11.4 ± 10.1	—	6.8 ± 8.9	—
RAAS inhibition	16 (100)	16 (100)	16 (100)	16 (100)
Diuretics	6 (37.5)	6 (37.5)	5 (31.3)	5 (31.3)
CCB	5 (31.3)	5 (31.3)	3 (18.8)	3 (18.8)
Statin	11 (68.8)	11 (68.8)	9 (56.3)	9 (56.3)
CVD	1 (6.3)	1 (6.3)	4 (25)	4 (25)
Clinical characteristics				
Hematocrit	0.38 ± 0.04	0.36 ± 0.04	0.39 ± 0.02	0.37 ± 0.03
HbA_{1c} (%)	7.31 ± 0.84	7.18 ± 0.97	7.18 ± 0.79	6.82 ± 0.82
HbA_{1c} (mmol/mol)	56 ± 9.2	55 ± 10.6	55 ± 8.6	51 ± 9.0
Creatinine (mmol/L)	68.6 ± 9.0	66.9 ± 7.5	70.6 ± 7.8	67.8 ± 8.9
eGFR (CKD-EPI) (mL/min/1.73 m^2)	94.2 ± 11.4	95.6 ± 12.3	94.1 ± 7.2	96.4 ± 6.7
BMI (kg/m^2)	30.22 ± 6.96	30.16 ± 7.02	31.69 ± 5.49	31.70 ± 5.63
SBP (mmHg)	124 ± 14	123 ± 14	123 ± 13	121 ± 9
DBP (mmHg)	71 ± 8	73 ± 7	71 ± 8	73 ± 7
HR (bpm)	74 ± 14	68 ± 12	74 ± 14	68 ± 12
MAP (mmHg)	86 ± 9	89 ± 8	87 ± 9	88 ± 7

Data are expressed as n (%), mean ± SD, or median (interquartile range). CCB, calcium channel blocker; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CVD, cardiovascular disease; DBP, diastolic blood pressure; eGFR, estimated GFR; HR, heart rate; MAP, mean arterial pressure.

Renal Tubular Effects

FE_{Na} was blunted with sitagliptin compared with placebo after single-dose administration (Fig. 1*A*) on experimental day 1 but not after 1 month of administration. In contrast, an increase in FE_{Na} was observed with sitagliptin compared with placebo at 3 h after 1 month of administration relative to after a single dose on experimental day 1 (Fig. 1*B*). No consistent change in FE_{Li} occurred during the study (Supplementary Fig. 3).

Renal and Systemic Hemodynamic Function Effects

Despite changes in natriuresis, acute and 1-month administration of sitagliptin did not influence renal hemodynamic function, blood pressure, or NICOM parameters (Supplementary Tables 2 and 3). A small but statistically significant increase in heart rate was observed with sitagliptin (+5.7 ± 3.3 vs. 2.2 ± 4.5 bpm, P = 0.019) compared with placebo after 1 month of administration at 3 h after study drug (Supplementary Table 3).

Plasma SDF-1α

Acute administration of sitagliptin increased circulating levels of intact SDF-$1\alpha^{1\text{-}67}$ compared with placebo after a single dose and after 1 month ($P < 0.0001$) (Fig. 2*A*). In contrast, truncated SDF-$1^{3\text{-}67}$ decreased acutely after single-dose sitagliptin administration compared with placebo ($P < 0.0001$) (Fig. 2*B*). Among subjects randomized to sitagliptin, plasma sitagliptin concentrations correlated with chronic percentage change in FE_{Na} and with SDF-$1\alpha^{1\text{-}67}$ concentrations, although these associations did not reach statistical significance (Supplementary Fig. 4*A* and *B*).

Neurohormones, Markers of Oxidative Stress, and Urinary Cytokines and Chemokines

Acute and 1-month sitagliptin administration did not change plasma ANP, plasma or urinary cGMP, plasma or urinary NO, or plasma renin or aldosterone concentrations compared with placebo (Supplementary Table 4). Urinary 8-isoprostane concentrations did not change in response to DPP-4 inhibition with sitagliptin; however, small but statistically significant increases in 8-hydroxy-2-deoxyguanosine concentrations were observed at 1 month (Supplementary Table 4). Most concentrations of urinary cytokines, chemokines, growth factors, and cognate receptors were unchanged after 1 month of sitagliptin compared with placebo (Supplementary Table 5). A small but statistically significant increase in interferon-γ was observed (Supplementary Table 5).

CONCLUSIONS

Although type 2 diabetes is predominantly recognized as a metabolic disease characterized by hyperglycemia and insulin resistance, the introduction of new medications, such as the SGLT2 inhibitors, GLP-1 receptor (GLP-1R) agonists,

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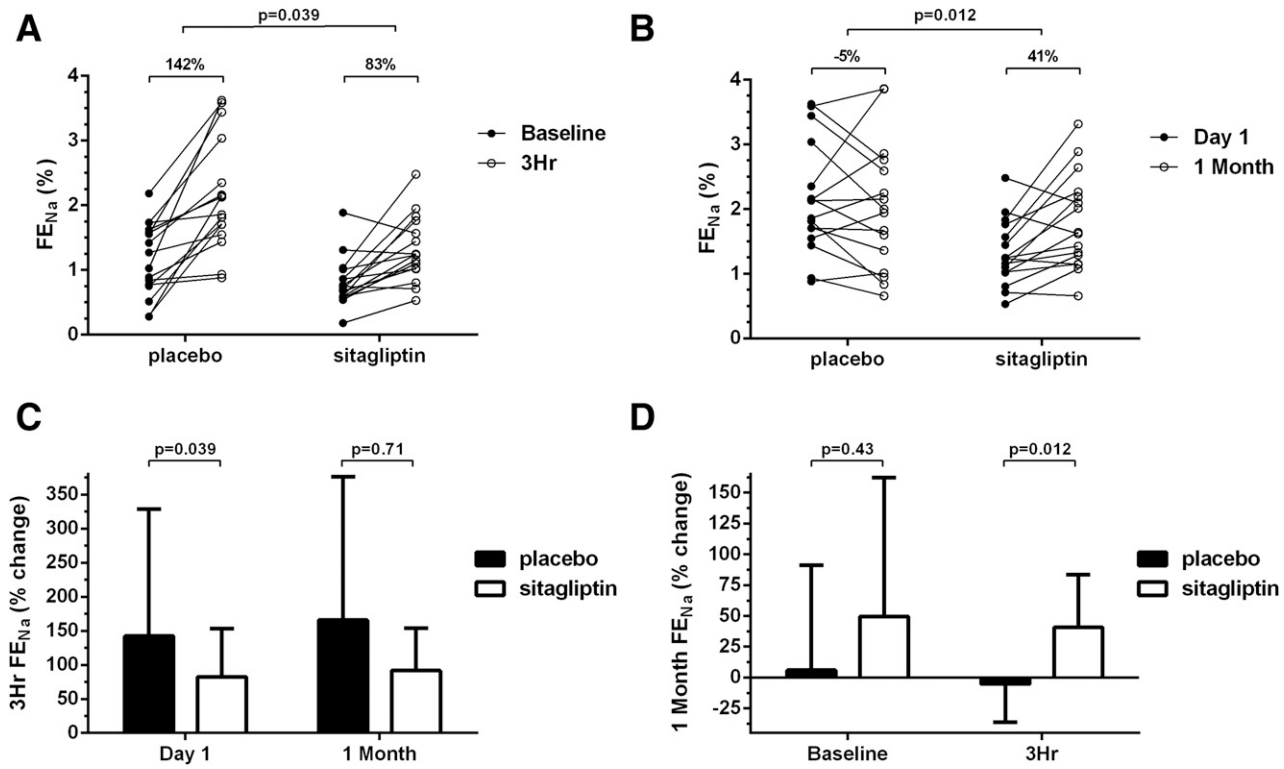

Figure 1—Acute and chronic changes in FE_{Na} in response to sitagliptin compared with placebo in patients with type 2 diabetes. *A*: FE_{Na} on day 1 at 3 h (compared with baseline) after the first dose of sitagliptin or placebo. *B*: FE_{Na} at 1 month, 3 h after sitagliptin or placebo (compared with day 1). *C*: Percentage change in FE_{Na} at 3 h (compared with baseline) on day 1 and after 1 month of sitagliptin or placebo. *D*: Percentage change in FE_{Na} at 1 month (compared with day 1) at baseline and at 3 h after sitagliptin or placebo. The horizontal bars in *A* and *B* indicate percentage changes in group means.

and DPP-4 inhibitors, has refocused attention on nonglycemic aspects of type 2 diabetes pathophysiology. Notably, the action of SGLT2 inhibitors has reemphasized the importance of exaggerated proximal tubular sodium reabsorption in type 2 diabetes. Furthermore, enhanced sodium absorption may lead to maladapted physiological responses within the diabetic kidney that contribute to diabetic nephropathy by promoting hyperfiltration and intraglomerular hypertension. Apart from systemic blood pressure control, blockade of the RAAS, and more recently, SGLT2 inhibition, few therapeutic strategies have attenuated diabetes-related complications in the kidney.

The demonstration that SGLT2 inhibition with empagliflozin in patients with type 2 diabetes and existing cardiovascular disease attenuates nephropathy (9,12, 21) has fostered interest in understanding whether and how other antihyperglycemic agents modify renal tubular and natriuresis-related pathways in the diabetic kidney (22). Although preclinical studies demonstrate that DPP-4 inhibition promotes natriuresis (23), evidence from human studies linking DPP-4 inhibition to natriuresis and changes in renal physiology is very limited (10). Here we describe several important features of sitagliptin action in the diabetic human kidney, with immediate clinical relevance.

First, sitagliptin increased urine FE_{Na} by 40%, findings detectable by 1 month of therapy. In contrast, FE_{Li}, a marker of proximal tubular sodium reabsorption, was unchanged, suggesting that sitagliptin induces natriuresis by blocking distal tubular sodium reabsorptive mechanisms rather than proximal sodium uptake, which is modulated by SGLT2 inhibitors. The increased FE_{Na} described here is consistent with a previous report that urinary sodium excretion increases by 2 weeks of sitagliptin therapy in subjects with type 2 diabetes (10). Second, we reveal, based on FE_{Li} studies, that these renal natriuretic effect(s) occur distal to the macula densa. Third, we show that natriuresis was acutely suppressed after 3 h after the first dose of sitagliptin, suggesting that initial sodium retention may occur with DPP-4 inhibitors in some individuals. Fourth, the initial acute suppression is transient and reverses over time, and natriuresis ultimately predominates after 1 month of treatment of sitagliptin. Fifth, despite changes in FE_{Na}, no changes in intrathoracic fluid volume, cardiac output, or systemic hemodynamic function were observed.

Agents that induce a proximal tubular natriuresis, such as SGLT2 inhibitors and carbonic anhydrase inhibitors, activate tubuloglomerular feedback, leading to afferent arteriolar vasoconstriction and reductions in GFR and ERPF (24). Conversely, agents that act distal to the macula densa are not coupled to these intrarenal autoregulatory mechanisms and should not affect tubuloglomerular feedback or renal hemodynamic function. Consistent with distal rather than proximal natriuretic pathways, we showed that renal hemodynamic function remained unchanged, both acutely and chronically, in response to sitagliptin. These findings have clinical relevance, implying that the focus of sitagliptin action is the distal tubule, thereby leaving tubuloglomerular feedback, afferent resistance, and intraglomerular pressure unaltered (24). In contrast, GLP-1R agonists

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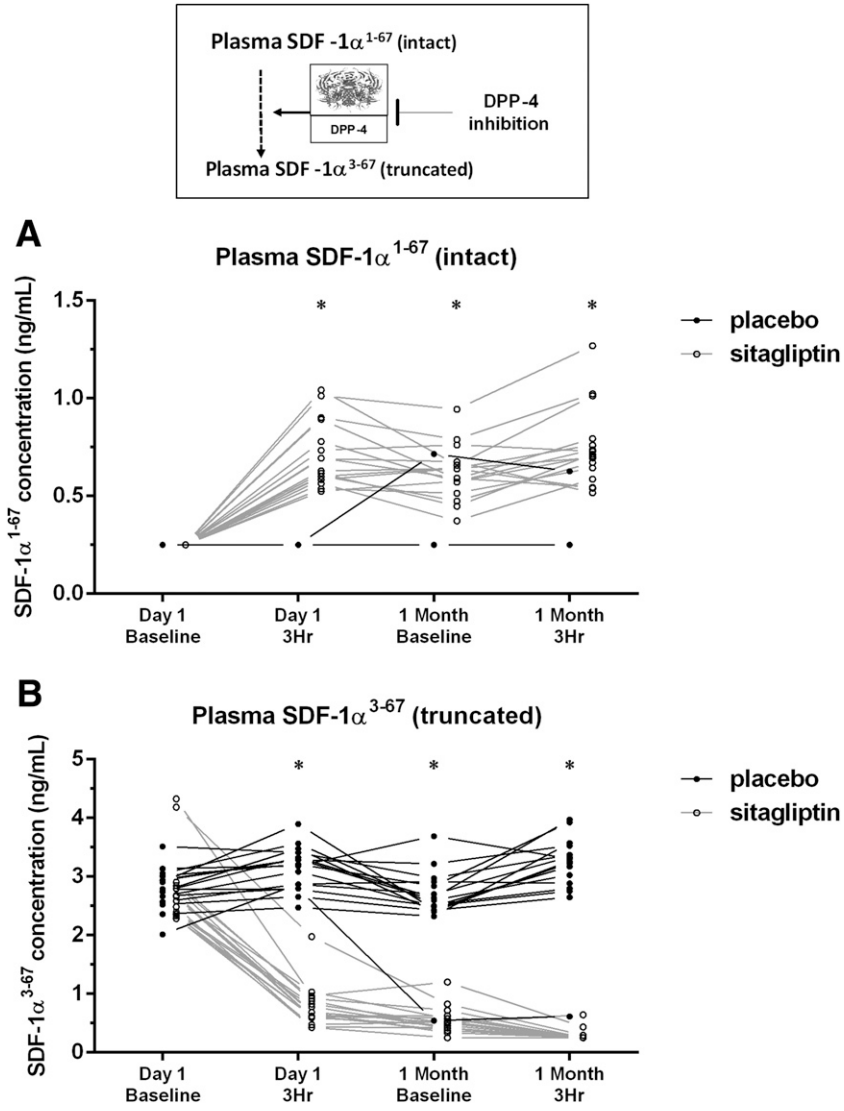

Figure 2—Immunoquantification of plasma SDF-1α in response to 1 month of sitagliptin administration compared with placebo in patients with type 2 diabetes. Individual patient data are indicated for immunoquantification of intact plasma SDF-1α$^{1-67}$ (*A*) and truncated plasma SDF-1α$^{3-67}$ (*B*) concentrations over the course of this 1-month clinical study in patients with type 2 diabetes administered placebo or sitagliptin. Inset (top) depicts metabolism of intact SDF-1α$^{1-67}$ to truncated SDF-1α$^{3-67}$ by the proteolytic actions of DPP-4 and suppression of DPP-4–mediated SDF-1α metabolism by DPP-4 inhibitors. *$P < 0.001$, sitagliptin vs. placebo.

attenuate diabetes-associated sodium avidity through distinct, more proximal mechanisms (16) and may therefore modify tubuloglomerular feedback. Consistent with this possibility, liraglutide reduced the composite renal microvascular outcome by 22% in patients with type 2 diabetes at heightened cardiovascular risk, an effect predominantly driven by reductions in macroalbuminuria (25).

Although SGLT2 inhibitors produce more robust natriuresis, blood pressure reduction, and potentially volume depletion, our current findings demonstrate that sitagliptin administration did not modify blood pressure or systemic hemodynamic parameters assessed by noninvasive cardiac monitoring. Hence, these physiological findings predict that DPP-4 inhibitors may exert clinically useful effects on renal hemodynamics in combination with SGLT2 inhibitors without the burden of excess volume contraction, hypotheses that should be carefully tested in future mechanistic human studies.

A major finding of clinical relevance is the demonstration that SDF-1α$^{1-67}$ is a physiological substrate of DPP-4 in humans with diabetes. SDF-1α$^{1-67}$, also known as C-X-C motif chemokine 12 (CXCL12) (4), is widely expressed and also localized to glomerular podocytes and distal tubular cells in mouse and human kidneys (26). We focused on assessment of SDF-1 because preclinical studies revealed that GLP-1R signaling does not mediate the natriuretic actions of DPP-4 inhibitors (20,23). SDF-1 expression is upregulated in the diabetic rat nephron (23), and SDF-1/CXCR4–dependent signaling suppresses renal oxidative stress and fibrosis through cAMP-mediated pathways. Here we applied immunocapture coupled with mass spectrometry to demonstrate, for the first time in humans with diabetes, that DPP-4 inhibition robustly increases intact plasma SDF-1α$^{1-67}$ and markedly decreases truncated SDF-1α$^{3-67}$. In preclinical studies, blockade of SDF-1α$^{1-67}$/CXCR4 signaling with the antagonist AMD 3100 reversed the natriuretic effects(s) of DPP-4 inhibition, mechanistically linking DPP-4 inhibition to enhanced urine sodium excretion via increased SDF-1α$^{1-67}$/CXCR4 signaling (23). Our findings associating increased levels of bioactive SDF-1α$^{1-67}$ with enhanced natriuresis in humans with diabetes treated with sitagliptin suggest conservation of biology across species and highlight SDF-1α$^{1-67}$ as a new mechanistic target for control of natriuresis in human subjects.

The extent of renal dysfunction and progression of nephropathy in subjects with diabetes has been partly linked to changes in oxidative stress, inflammation, or enhanced growth factor activity (27). Although DPP-4 inhibitors have been reported to exert anti-inflammatory effects in preclinical and clinical studies (4,28), sitagliptin therapy was not associated with changes in urinary inflammatory cytokines, chemokines, growth factors, or markers of oxidative stress (isoprostane), aside from small changes in 8-hydroxy-2-gaunosine and interferon-γ at 1 month. Hence, although we cannot exclude the possibility that DPP-4 inhibition may produce chronic anti-inflammatory actions in longer studies or reduce inflammation in different tissue compartments from unique patient populations, the current data suggest that clinically detectable changes in inflammatory status or oxidative stress are not a prominent feature of short-term therapy with DPP-4 inhibition in patients with type 2 diabetes.

The lack of effect of DPP-4 inhibitors on renal function in large cardiovascular

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outcome studies is consistent with our short-term study results, because modification of distal tubular natriuresis via DPP-4 inhibition should not affect renal function or tubuloglomerular feedback pathways. Furthermore, the clinical relevance of our findings is highlighted by the increasing use of DPP-4 inhibitors and SGLT2 inhibitors in combination therapy for type 2 diabetes. Both drugs modify renal tubular sodium handling, with potential implications for renal and systemic hemodynamic function. Our current findings localize renal natriuretic effects of DPP-4 inhibition to the distal convoluted tubule. Hence, we predict that DPP-4 inhibitors should not interfere with or diminish the effects of other drugs that block proximal renal tubular sodium reabsorption. Further mechanistic studies examining the simultaneous renal tubular

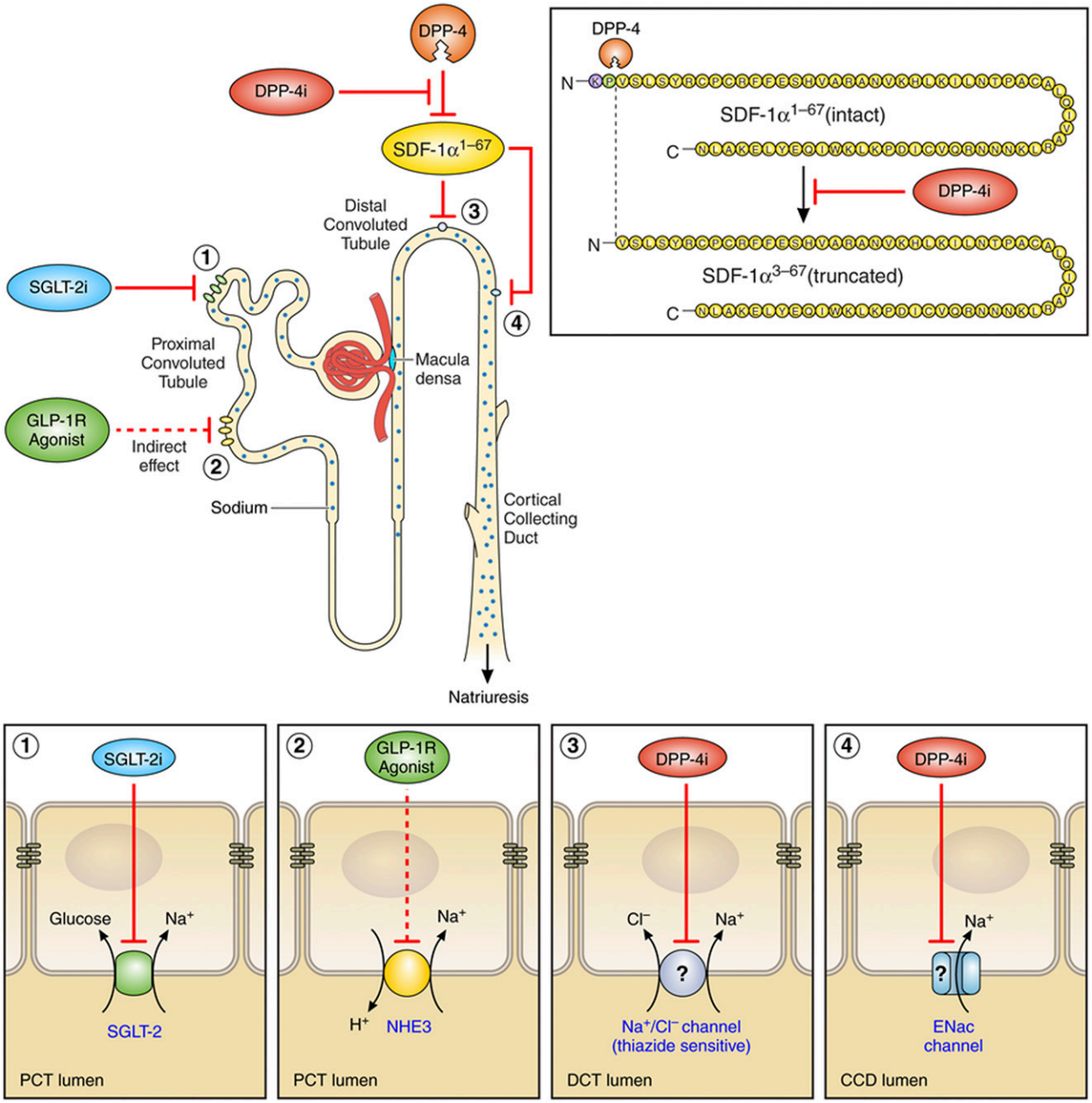

Figure 3—Putative natriuretic mechanisms for DPP-4 inhibitors (DPP-4i). DPP-4i promote a distal tubular natriuresis that does not affect renal or systemic hemodynamic function in patients with type 2 diabetes without nephropathy. Although these DPP-4i–mediated natriuretic mechanisms remain incompletely understood, they occur in conjunction with a significant increase in circulating intact SDF-1α^{1-67} and suppression of truncated SDF-1α^{3-67}. Physiologically, SDF-1α^{1-67} is a substrate of the DPP-4 enzyme. Administration of DPP-4i in patients with type 2 diabetes prevents the N-terminal cleavage and inactivation of intact plasma SDF-1α^{1-67} to truncated plasma SDF-1α^{3-67} (inset) by inhibiting the proteolytic activity of DPP-4. Potential distal tubular ion transport channels that may link DPP-4 inhibition to stimulating distal natriuresis include the Na^+/Cl^- thiazide-sensitive channel (*3*), and the epithelial sodium channel (ENac) (*4*). In contrast, SGLT2 inhibitors (SGLT2i) (*1*) and GLP-1RA (*2*) inhibit more proximal sodium reabsorption in the proximal convoluted tubules (PCTs) and influence tubuloglomerular feedback by increasing sodium delivery at the macula densa. SGLT2i modify renal hemodynamic function. CCD, cortical collecting duct; DCT, distal convoluted tubule.

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response(s) to coadministered SGLT2 inhibitors and DPP-4 inhibitors will further clarify the mechanistic rationale for combination therapy in the treatment of type 2 diabetes.

The current study is not without limitations. The study was adequately powered to determine the effect of sitagliptin on FE_{Na}, but the small sample size limited our ability to perform in-depth analyses around clinical factors such as duration of diabetes, degree of albuminuria, or the effect of coadministration of other drugs. We attempted to reduce variability by using a placebo-controlled study design that included a prestudy protocol specifying limits around dietary factors that can influence GFR, including dietary protein content, dietary sodium consumption, background RAAS inhibition, and ambient glucose levels. Furthermore, there are currently no available assays to measure tubuloglomerular feedback in humans aside from measuring changes in renal function in response to changes in tubular sodium delivery; therefore, we were unable to directly quantify the effect of sitagliptin on tubuloglomerular feedback.

In conclusion, DPP-4 inhibition promotes a distal tubular natriuresis after short-term therapy that does not modify renal or systemic hemodynamic function (Fig. 3). Sitagliptin administration increased plasma levels of SDF-$1\alpha^{1\text{-}67}$, a chemokine that promotes natriuresis in preclinical studies. We conclude that SDF-$1\alpha^{1\text{-}67}$ is a strong candidate effector that mediates the natriuretic effect(s) of sitagliptin, highlighting potentiation of SDF-1 activity as a new focus for therapeutic strategies targeting natriuresis. Our current studies predict that combination therapy with SGLT2 inhibitors and DPP-4 inhibitors should not abrogate the beneficial renal effects observed with SGLT2 inhibitors alone, findings with implications for the treatment of type 2 diabetes.

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Author Contributions. J.A.L., H.R., Y.L., and V.L. conducted the study and performed the measurements. J.A.L., D.J.D., and D.Z.I.C. designed and planned the entire study, interpreted the data, discussed the intellectual content, and wrote the manuscript. J.A.L. and D.Z.I.C. served as the study investigators and were present at and supervised all clinical visits during the study. H.R., Y.L., S.K., R.A., A.L., and V.L. critically reviewed the manuscript. L.E.L. was an independent statistician who performed the statistical analysis and contributed to the graphical representation of the data. S.K., R.A., and A.L. helped with data collection and data management. H.H., L.H., and W.W. performed the SDF-1 and sitagliptin assays and contributed to the critical review of the manuscript. D.Z.I.C. 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.

References

1. Cornel JH, Bakris GL, Stevens SR, et al.; TECOS Study Group. Effect of sitagliptin on kidney function and respective cardiovascular outcomes in type 2 diabetes: outcomes from TECOS. Diabetes Care 2016;39:2304–2310

2. Turner LW, Nartey D, Stafford RS, Singh S, Alexander GC. Ambulatory treatment of type 2 diabetes in the U.S., 1997-2012. Diabetes Care 2014;37:985–992

3. Lovshin JA, Drucker DJ. Incretin-based therapies for type 2 diabetes mellitus. Nat Rev Endocrinol 2009;5:262–269

4. Mulvihill EE, Drucker DJ. Pharmacology, physiology, and mechanisms of action of dipeptidyl peptidase-4 inhibitors. Endocr Rev 2014;35:992–1019

5. Green JB, Bethel MA, Armstrong PW, et al.; TECOS Study Group. Effect of sitagliptin on cardiovascular outcomes in type 2 diabetes. N Engl J Med 2015;373:232–242

6. White WB, Cannon CP, Heller SR, et al.; EXAMINE Investigators. Alogliptin after acute coronary syndrome in patients with type 2 diabetes. N Engl J Med 2013;369:1327–1335

7. Scirica BM, Bhatt DL, Braunwald E, et al.; SAVOR-TIMI 53 Steering Committee and Investigators. Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. N Engl J Med 2013;369:1317–1326

8. U.S. Food and Drug Administration. FDA Drug and Safety Communication: FDA adds warnings about heart failure risk to labels of type 2 diabetes medicines containing saxagliptin and alogliptin, 2016. Available from https://www.fda.gov/Drugs/DrugSafety/ucm486096.htm. Accessed 12 May 2017

9. Zinman B, Lachin JM, Inzucchi SE. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. N Engl J Med 2016;374:1092–1094

10. Tonneijck L, Smits MM, Muskiet MH, et al. Renal effects of DPP-4 inhibitor sitagliptin or GLP-1 receptor agonist liraglutide in overweight patients with type 2 diabetes: a 12-week, randomized, double-blind, placebo-controlled trial. Diabetes Care 2016;39:2042–2050

11. Schnurr E, Lahme W, Küppers H. Measurement of renal clearance of inulin and PAH in the steady state without urine collection. Clin Nephrol 1980;13:26–29

12. Cherney DZI, Perkins BA, Soleymanlou N, et al. Renal hemodynamic effect of sodium-glucose cotransporter 2 inhibition in patients with type 1 diabetes mellitus. Circulation 2014;129:587–597

13. Gomez DM. Evaluation of renal resistances, with special reference to changes in essential hypertension. J Clin Invest 1951;30:1143–1155

14. Bjornstad P, Škrtić M, Lytvyn Y, Maahs DM, Johnson RJ, Cherney DZ. The Gomez equations and renal hemodynamic function in kidney disease research. Am J Physiol Renal Physiol 2016;311:F967–F975.

15. Cherney DZ, Reich HN, Scholey JW, et al. The effect of aliskiren on urinary cytokine/chemokine responses to clamped hyperglycaemia in type 1 diabetes. Diabetologia 2013;56:2308–2317

16. Lovshin JA, Barnie A, DeAlmeida A, Logan A, Zinman B, Drucker DJ. Liraglutide promotes natriuresis but does not increase circulating levels of atrial natriuretic peptide in hypertensive subjects with type 2 diabetes. Diabetes Care 2015;38:132–139

17. Cherney DZ, Reich HN, Jiang S, et al. Hyperfiltration and effect of nitric oxide inhibition on renal and endothelial function in humans with uncomplicated type 1 diabetes mellitus. Am J Physiol Regul Integr Comp Physiol 2012;303:R710–R718

18. Wang W, Choi BK, Li W, et al. Quantification of intact and truncated stromal cell-derived factor-1α in circulation by immunoaffinity enrichment and tandem mass spectrometry. J Am Soc Mass Spectrom 2014;25:614–625

19. Zeng W, Xu Y, Constanzer M, Woolf EJ. Determination of sitagliptin in human plasma using protein precipitation and tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2010;878:1817–1823

20. Rieg T, Gerasimova M, Murray F, et al. Natriuretic effect by exendin-4, but not the DPP-4 inhibitor alogliptin, is mediated via the GLP-1

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receptor and preserved in obese type 2 diabetic mice. Am J Physiol Renal Physiol 2012;303:F963–F971
21. Anders HJ, Davis JM, Thurau K. Nephron protection in diabetic kidney disease. N Engl J Med 2016;375:2096–2098
22. Perkins BA, Udell JA, Cherney DZ. No need to sugarcoat the message: is cardiovascular risk reduction from SGLT2 inhibition related to natriuresis? Am J Kidney Dis 2016;68:349–352
23. Takashima S, Fujita H, Fujishima H, et al. Stromal cell-derived factor-1 is upregulated by dipeptidyl peptidase-4 inhibition and has protective roles in progressive diabetic nephropathy. Kidney Int 2016;90:783–796
24. Heerspink HJ, Perkins BA, Fitchett DH, Husain M, Cherney DZ. Sodium glucose cotransporter 2 inhibitors in the treatment of diabetes mellitus: cardiovascular and kidney effects, potential mechanisms, and clinical applications. Circulation 2016;134:752–772
25. Marso SP, Daniels GH, Brown-Frandsen K, et al.; LEADER Steering Committee; LEADER Trial Investigators. Liraglutide and cardiovascular outcomes in type 2 diabetes. N Engl J Med 2016;375:311–322
26. Chen LH, Advani SL, Thai K, et al. SDF-1/CXCR4 signaling preserves microvascular integrity and renal function in chronic kidney disease. PLoS One 2014;9:e92227
27. Navarro-González JF, Mora-Fernández C. The role of inflammatory cytokines in diabetic nephropathy. J Am Soc Nephrol 2008;19:433–442
28. Makdissi A, Ghanim H, Vora M, et al. Sitagliptin exerts an antinflammatory action. J Clin Endocrinol Metab 2012;97:3333–3341

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