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Effect of immediate and prolonged GLP-1 receptor agonist administration on uric acid and kidney clearance: *post-hoc* analyses of four clinical trials

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Abstract

Aims

To determine the effects of glucagon-like peptide (GLP)-1 receptor agonists (RA) on uric acid (UA) levels and kidney UA clearance.

Material and methods

This study involved post-hoc analyses of 4 controlled clinical trials, which assessed actions of GLP-1RA administration on kidney physiology. The immediate effects of GLP-1RA exenatide infusion vs placebo were determined in 9 healthy overweight men (Study-A) and in 52 overweight T2DM patients (Study-B). The effects of 12 weeks of long-acting GLP-1RA liraglutide vs placebo in 36 overweight T2DM patients (Study-C) and of 8 weeks of short-acting GLP-1RA lixisenatide vs once-daily titrated insulin glulisine in 35 overweight T2DM patients (Study-D) were also examined. Plasma UA, fractional (inulin-corrected) and absolute urinary excretion of UA (UE_{UA}) and sodium (UE_{Na}), and urine pH were determined.

Results

Median baseline plasma UA level was 5.39 to 6.33 mg/dL across all studies (17%-22% of subjects were hyperuricaemic). In Study-A, exenatide infusion slightly increased plasma UA (+0.07 ± 0.02 mg/dL, p=0.04), and raised absolute-UE_{UA} (+1.58 ± 0.65 mg/min/1.73 m^2, p=0.02), but did not affect fractional UE_{UA} compared to placebo. Fractional UE_{UA} and absolute UE_{UA} correlated with increases in urine pH (r: 0.86, p=0 .003 and r:0.92, p<0.001, respectively). Fractional UE_{UA} correlated with increased fractional UE_{Na} (r: 0.76, p=0.02). In Study-B, exenatide infusion did not affect plasma UA, but increased fractional UE_{UA} (+0.76 ± 0.38%, p=0.049) and absolute UE_{UA} (+0.75 ± 0.27 mg/min/1.73 m^2, p=0.007), compared to placebo. In regression analyses, both parameters were explained by changes in urine pH and, in part, by changes in UE_{Na}. In Study-C, liraglutide treatment did not affect plasma UA, UE_{UA}, UE_{Na} or urine pH, compared to placebo. In Study-D, lixisenatide treatment increased UE_{Na} and urine pH from baseline, but did not affect plasma UA or UE_{UA}.

Conclusion

Immediate exenatide infusion increases UE_{UA} in overweight healthy men and in T2DM patients, probably by inhibiting Na^+/H^+-exchanger type-3 in the renal proximal tubule. Prolonged treatment with a long-acting or short-acting GLP-1RA does not affect plasma UA or UE_{UA} in T2DM patients with normal plasma UA levels and at relatively low cardiovascular risk. Our results suggest that the cardio-renal benefits of GLP-1RA are not mediated through changes in UA.

Introduction

In humans, uric acid (UA) is the final breakdown product of purine nucleotides, which originate from exogenous dietary sources, and from endogenous de novo synthesis and tissue catabolism.[1,2] UA formation from purines occurs mainly in the liver, with a prominent role for xanthine oxidase, the rate-limiting enzyme in UA biosynthesis.[3,4] Normally, >70% of UA is excreted into the urine, with a fractional excretion of 5% to 10%, while the remainder is excreted through the intestines and perspiration.[3,5,6] Hyperuricaemia is usually defined as a plasma UA level > 6.8 mg/dL (405 μmol/L, corresponding with its solubility point)[6] and can be caused by reduced excretion by the kidney, accounting for 90% of cases of hyperuricaemia, or by UA over-production in the liver.[3] Hyperuricaemia can result in deposition of UA crystals, leading to gout and nephrolithiasis. Moreover, evidence suggests that increased plasma UA levels, although in the high normal range,[4,7] are an independent risk factor for impaired fasting glucose, diabetes mellitus, hypertension, heart failure, coronary heart disease and chronic kidney disease.[8] Accordingly, the indication for plasma UA-lowering therapy, with either xanthine-oxidase inhibitors or uricosuric agents, may be broader than that for gout or UA nephrolithiasis. Interestingly, in studies in diverse populations including type 2 diabetes mellitus (T2DM) patients,[4,9-11] plasma UA-lowering therapy improved surrogate cardio-renal end points, although dedicated large-sized trials, such as the ongoing ALL-HEART study (ISRCTN32017426), are needed to firmly establish causation between UA lowering and long-term clinical outcomes.[4]

Over the last 3 decades, multiple antihyperglycaemic drug classes, with different modes of action, have been successfully licensed for the treatment of hyperglycaemia in T2DM. The results of recent long-term cardiovascular safety trials in thousands of at-risk T2DM patients indicate the beneficial effect of 2 of these drug classes on cardiovascular and renal events and mortality: (1) glucagon-like peptide-1 (GLP-1) receptor agonists (RA) (ie, liraglutide[12,13] and semaglutide,[14] but not exenatide once-weekly, which tended [p=0.06] to reduce cardiovascular risk[15]) and (2) sodium-glucose co-transporter-2 (SGLT2) inhibitors (ie, empagliflozin[16,17] and canagliflozin[18]). Delineation of the underlying cardio-renal protective mechanisms may have immediate relevance for prevention and treatment of diabetes-related complications, but many uncertainties remain. For SGLT2 inhibitors, some have suggested that benefits are explained, in part, by their ability to reduce serum UA levels, probably through increased urinary UA excretion.[19] The effects of GLP-1RAs on plasma UA levels and urinary excretion of UA (UE_{UA}) in humans are hitherto unknown. In rats with streptozotocin-induced (type 1) diabetes, daily GLP-1-peptide injections for 10 weeks reduced serum UA concentrations by 58%, compared to untreated diabetic rats.[20]

As GLP-1RA administration inhibits Na^+/H^+-exchanger type-3 (NHE3) activity in the proximal tubule, causing natriuresis and alkalisation of the urine,[21-24] we hypothesise that this antihyperglycaemic drug class may reduce plasma UA levels by increasing urinary UA excretion in humans, those with and those without T2DM.[25-27] To explore the effects of currently available GLP-1RAs, with clinically relevant differences in their pharmacokinetic

profile (see below), on plasma UA and UE_{UA}, we performed a *post-hoc* analysis of 4 clinical trials that assessed the effects of these drugs on kidney physiology.[21,22,24,28] First, we assessed the immediate effects of intravenous administration of exenatide in healthy overweight men and T2DM patients. Second, as immediate effects on tubular UA handling could be offset during chronic intervention, we also examined the effects of prolonged GLP-1RA therapy in overweight T2DM patients. The pharmacokinetic profile of a GLP-1RA is known to have different effects on the rate of gastric emptying, urinary sodium excretion and urinary alkalization during prolonged administration.[22,28-30]

Material and methods

Trial designs

The trials involved *post-hoc* analyses of 4 phase-4 clinical intervention trials, all conducted at the Diabetes Center of the VU University Medical Center (VUMC) in Amsterdam between May 2013 and April 2017. All trials were designed primarily to assess the effects of immediate or prolonged GLP-1RA-administration on kidney physiology in overweight healthy men and T2DM patients.

A detailed overview of trial designs and testing procedures is shown in Figure 1. Study-A was an acute, non-blinded, placebo-controlled trial, examining the effect of placebo (isotonic saline) and subsequent exenatide infusion at therapeutic concentrations in healthy overweight men.[21] Study-B was an acute, randomised, double-blind, placebo-controlled, parallel-group trial, examining the effect of exenatide infusion in T2DM patients.[24] Patients from Study-B were subsequently enrolled in Study-C, which was a 12-week, randomised, double-blind, placebo-controlled trial, examining the effect of subcutaneous injections with liraglutide in T2DM patients.[28] Study-D was an 8-week, randomised, open-label, comparator-controlled trial, examining the effect of subcutaneous injections with lixisenatide vs once-daily titrated insulin glulisine in T2DM patients.[23]

All trials were registered at ClinicalTrials.gov (ID's: NCT01744236 [Study-A–Study-C] and NCT02276196 [Study-D]). Trials were approved by the local ethics review board, and were conducted in accordance with the Declaration of Helsinki and International Conference on Harmonization on Good Clinical Practice. All subjects provided written informed consent before any trial-related activities. Detailed descriptions of participants, interventions and randomisation procedures have been reported previously.[21,23,24,28,31] A brief description is provided online in the Supplemental methods.

Study protocols

Two days prior to testing procedures, participants were instructed to adhere to a controlled intake of protein (1.5-2.0 g/kg/d) and of sodium (~150-200 mmol/d). In addition, prior to experiments, participants were instructed to refrain from vigorous physical activity and from alcohol consumption for ≥24 hours, and to avoid caffeine-containing products or nicotine for ≥12 hours. After an overnight fast, and before arriving at the CRU of the Diabetes Center VUMC

at 7:30 AM, patients consumed 500 mL of tap water to stimulate diuresis. T2DM patients delayed morning medications, with the exception of metformin and thyroid hormone replacement therapy. Participants assumed a semi-recumbent position in a temperature-controlled room (23.0 ± 1.0°C) throughout testing procedures. Intravenous catheters were inserted in an antecubital vein of both forearms to allow intermittent blood sampling (non-dominant side) and infusion of kidney tracer substances (dominant side). Blood and urine were obtained for outcome variables as previously described in detail. [21,23,24,28] Subsequently, kidney tests commenced as previously described in detail.[21,23,24,28] In all studies, a bolus of inulin was administered over 10 minutes, which was followed by a continuous infusion over 90 minutes; this served as an equilibration period.

In Study-A, subjects subsequently underwent 2 consecutive urine collection periods of 45 minutes during placebo infusion, which were repeated after 60 minutes of infusion with exenatide (Figure 1).[21] In Study-B, participants subsequently underwent 2 urine collection periods of 45 minutes at baseline, which were repeated after 60 minutes of infusion with exenatide or placebo (Figure 1).[24] In Study-C, the baseline measurements of Study-B were repeated after 12 weeks of treatment with liraglutide or placebo (Figure 1).[28] In Study-D, participants consumed a standardized mixed breakfast consisting of bread with cheese and jam, and milk (416 kcal; 22 g protein, 48 g carbohydrates and 14 g fat) 35 minutes after initiation of the inulin infusion (Figure 1).[23] After the equilibration period, patients underwent 2 urine collection periods of 45 minutes at baseline, which were repeated at week 8. After 8 weeks, lixisenatide or insulin glulisine was administered 30 and 10 minutes, respectively, before the standardized breakfast.

In all studies, diuresis was prompted by oral intake of 10 mL/kg (maximum 1000 mL) tap water during the inulin equilibration period, followed by an intake of 200 mL/h for the remainder of the testing day. Before and after each urine collection period, blood samples were taken to obtain outcome variables as described previously in detail.[21,23,24,28] UA-excretion was measured over the second of 2 consecutive urine collection periods in Study-A–Study-C, and over both of the 2 urine collection periods in Study-D.

Biochemical analyses

UA was measured as urate, using an enzymatic colorimetric test (Cobas-C501, Roche Diagnostics, Indianapolis, Indiana) and urine pH was buffered to >8.0 with NaOH. Inulin was analysed as described previously.[24] Routine blood determinations (HbA_{1c}, glucose, sodium, osmolality and creatinine) were performed at baseline and after prolonged therapy, using conventional assay methods, by the Department of Clinical Chemistry at the VUMC. Venous blood glucose was measured throughout the kidney tests using an YSI-2300 STAT glucose analyser (YSI Life Sciences, Yellow Springs, Ohio). Insulin was determined using an immunometric assay (ADVIA Centaur-XP Immunoassay System, Siemens Healthcare, Erlangen, Germany). Cross-reactivity of the assay with insulin glulisine (low) and insulin glargine (high) has been described.[22] Urine pH was measured by a hand-held VARIO 2V00 pH meter and SenTix-V electrode (Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany).

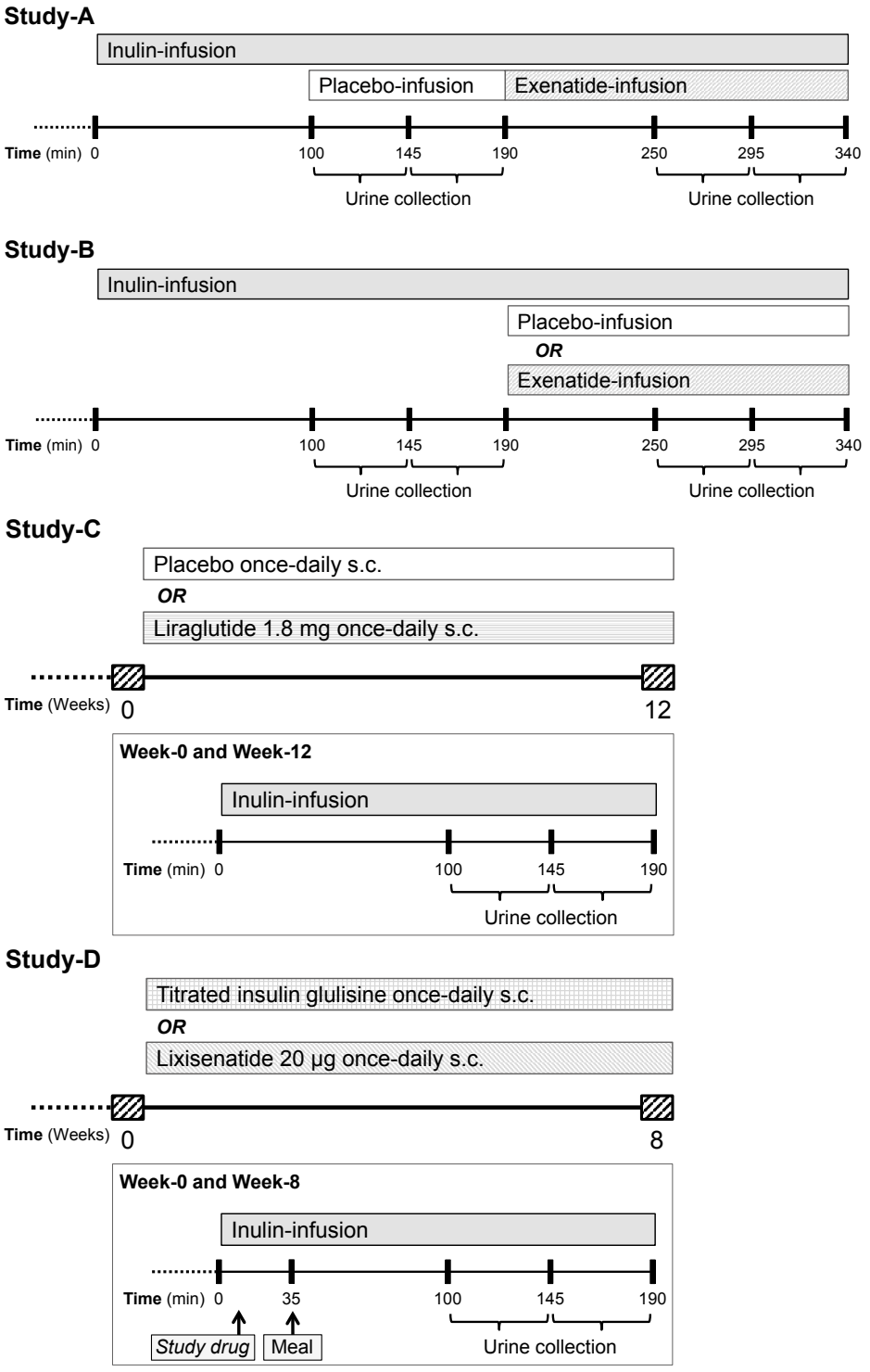

Figure 1. Trial designs

Outcomes

In all studies, plasma UA, fractional and absolute UE of UA (UE_{UA}) and sodium (UE_{Na}), GFR, urine pH, urine osmolality, insulin (serum or plasma) and glucose (blood or plasma) were measured. Urinary glucose was determined in Study-B–Study-D. In Study-C, fractional UE_{UA} was additionally assessed at weeks 0, 2 and 12.

Calculations

Fractional UE_{UA} and UE_{Na} were calculated by using inulin as a reference substance, unless stated otherwise. Absolute electrolyte excretions were calculated by multiplying urinary electrolyte concentrations by urine flow. GFR was calculated from inulin clearances. GFR and absolute electrolyte excretion were corrected for body surface area using the Mosteller formula.[21,23,24,28]

Statistical methods

No *a priori* sample-size power calculation was performed for these *post-hoc* exploratory analyses. Statistical analyses were performed in the per protocol population of the separate trials as described previously,[21,23,24,28] using SPSS Statistics for Windows, V22.0 (IBM Corp, Armonk, New York). Log10-transformation of non-Gaussian distributed data was carried out to achieve normality prior to analyses. Paired *t*-test or Wilcoxon signed-rank test was used as appropriate to identify single effects of exenatide vs placebo in Study-A and to make within-group comparisons in Study-D. Multivariable linear regression models, adjusting for corresponding baseline values only, were used to identify the effects of exenatide vs placebo (Study-B), liraglutide vs placebo (Study-C) and lixisenatide vs insulin glulisine (Study-D). The Mann-Whitney test was used to assess between-group differences in urinary glucose in Study-D. Pearson's correlation or Spearman signed rank test was used as appropriate to assess associations. A two-sided p- value <0.05 was considered statically significant for all comparisons. Data are presented as least square mean ± SEM or, in the case of a non-Gaussian distribution, as median (interquartile range), unless stated otherwise.

Results

Study-A

The study population comprised 9 subjects (Table 1).[21] Plasma UA was 6.33 (6.05-7.05) mg/dL (2 subjects were hyperuricaemic) during placebo infusion, and increased upon exenatide infusion to 6.48 (6.09-7.08) mg/dL (+0.07 ± 0.02 mg/dL, p=0.04). Exenatide did not significantly affect fractional UE_{UA} (+0.82 ± 0.47%, p=0.2), but increased absolute UE_{UA} compared to placebo infusion (+1.58 ± 0.65 mg/min/1.73 m^2, p=0.02) (Figure 2). As previously reported, exenatide increased GFR, fractional UE_{Na}, absolute UE_{Na}, urine pH (from 6.53 (6.03-6.90) to 7.04 (6.89-7.34), p=.005) and osmolality (Supplemental Table 1).[21] Exenatide reduced plasma glucose, without affecting plasma insulin concentrations (Supplemental Table 1).[21]

Changes in fractional UE_{UA} and absolute UE_{UA} correlated with changes in urine pH (r: 0.90, p=0.001 and r: 0.82, p=0.007, respectively) (Figure 3A-B), but not with changes in plasma

UA (r: −0.20, p=0.6 and r: −0.35, p=0.4), in glucose (r: 0.58, p=0.1 and r: 0.27, p=0.5) or in insulin (r: 0.17, p=0.7 and r: 0.26, p=0.5). Changes in fractional UE_{UA} correlated with changes in fractional UE_{Na} (r: 0.75, p=0.019) (Figure 3C). Changes in fractional UE_{UA}, absolute UE_{UA} and plasma UA did not correlate with baseline plasma UA (all p>0.3).

Study-B

The study population comprised 52 patients (Table 1).[24] Mean ± SD baseline UA was 5.50 ± 1.04 mg/dL (9 patients were hyperuricaemic) and did not change during immediate exenatide-infusion compared to placebo (−0.01 ± 0.02 mg/dL, p=0.8). While UA levels were not affected, exenatide-infusion increased fractional UE_{UA} (+0.76 ± 0.38%, p=0.049) and absolute UE_{UA} (+0.75 ± 0.27 mg/min/1.73 m^2, p=0.007), compared to placebo (Figure 2). As previously reported, exenatide increased fractional UE_{Na}, absolute UE_{Na}, urine pH (+0.72 ± 0.11, p<0.001) and urinary osmolality (Supplemental Table 2).[24] Urinary glucose at baseline was <0.11 mmol/L in all but one patient (0.97 mmol/L) and was not affected by exenatide.[24] Exenatide infusion reduced blood glucose during kidney tests, and increased serum insulin concentrations compared to placebo (Supplemental Table 2). There were no changes in plasma renin concentrations (PRC).[24]

In regression analyses, exenatide-induced alterations in fractional UE_{UA} and absolute UE_{UA} were largely explained by the change in urine pH (regression coefficient reduced to 0.01 ± 0.45% [p=0.9] and to −0.03 ± 0.28 mg/min/1.73 m^2 [p=0.9], respectively) and, in part, by respective changes in fractional UE_{Na} (to 0.58 ± 0.37%, p=0.1) and absolute UE_{Na} (to 0.50 ± 0.25 mg/min/1.73 m^2, p=0.049). Correcting for the change in blood glucose partially lowered the effect of exenatide on UE_{UA} (to 0.67 ± 0.64% [p=0.3] and to 0.56 ± 0.48 mg/min/1.73 m^2 [p=0.3]). When correcting for exenatide-induced change in insulin, the regression coefficient of fractional UE_{UA} and absolute UE_{UA} increased to 1.11 ± 0.45% (p=0.02) and 0.95 ± 0.31 mg/min/1.73 m^2 (p=0.004), respectively. In the exenatide arm, changes in fractional UE_{UA}, absolute UE_{UA} and plasma UA did not correlate with baseline UA levels (all p>0 .6).

Study-C

The study population comprised 36 patients (Table 1).[28] Mean ± SD baseline UA was 5.39 ± 1.07 mg/dL (6 patients were hyperuricaemic). After a 12-week treatment with liraglutide, no alterations in UA (p=0.8) (Figure 4A) or in UE_{UA} (Figure 2 and Figure 4B) were observed compared to placebo. As previously reported, GFR, UE_{Na}, PRC, urine pH, urine osmolality and urinary glucose excretion were not affected after 12 weeks of treatment (Supplemental Table 3).[28] Yet, compared to placebo, liraglutide reduced HbA_{1c} by 1.3 ± 0.2% (p=0.001), plasma glucose by 1.3 ± 0.3 mmol/L (p<0.001) and body weight by 1.9 ± 0.7 kg (p=0.009).[28] There were no changes in PRC.[28] At Week 2, no changes in UA (p=0.8), creatinine-based fractional UE_{UA} (−1.55 ± 0.88%, p=0.09) or fractional UE_{Na} were observed in the liraglutide arm compared to the placebo arm (Figure 4A-B).[28] In the liraglutide arm, but not in the placebo arm, changes from baseline to Week 12 in plasma UA correlated with baseline plasma UA (r: −0.52, p=0.022)

Table 1. Baseline clinical and biochemical characteristics

	Study-A (N=9)	Study-B (N=52)	Study-C (N=36)	Study-D (N=35)
Clinical characteristics				
Male sex, *n* (%)	9 (100)	39 (77)	27 (75)	23 (66)
Age, years	22.0 [22.0–23.0]	62.9 ± 6.9	63.0 ± 7.0	61.7 ± 6.6
Weight, kg	96.0 (7.0)	99.0 ± 14.3	101.2 ± 14.9	97.5 ± 16.4
BMI, kg/m^2	29.3 ± (1.7)	31.1 [28.3–33.6]	31.2 [29.2–33.3]	31.5 ± 4.0
Systolic BP, mm Hg	113 [107–123]	135 ± 15	137 ± 16	134 ±16
Diastolic BP, mm Hg	67 [61–71]	76 ± 6	77 ± 6	76 ± 9
Biochemistry				
HbA_{1c}, %	5.1 [5.0–5.3]	7.3 ± 0.6	7.4 ± 0.7	8.0 ± 0.9
HbA_{1c}, mmol/mol	32 [31–34]	57 ± 7	58 ± 8	64 ± 9
Fasting plasma glucose, mmol/L	4.9 [4.4–5.2]	8.4 ± 1.5	8.6 ± 1.7	7.0 ± 2.0
HOMA2-IR	0.67 [0.59–1.47]	1.7 [1.2–2.5]	1.6 [1.1–2.4]	N/A[a]
Plasma-UA, mg/dL	6.33 [6.05-7.05]	5.50±1.04	5.39±1.07	5.68±1.19
Hyperuricemia[b], *n* (%)	2 (22)	9 (17)	6 (17)	7 (20)
Diabetes history characteristics				
Type 2 diabetes duration, years	N/A	7 [4–12]	8 [4–12]	13 ± 7
Metformin use, *n* (%)	N/A	49 (94)	34 (94)	32 (91)
Sulphonylurea use, *n* (%)	N/A	23 (44)	15 (42)	0 (0)
Insulin use, *n* (%)	N/A	0 (0)	0 (0)	35 (100)
Xanthine-oxidase inhibitor use, *n* (%)	0 (0)	1 (2)	1 (3)	2 (6)
Antihypertensive medication use, *n* (%)	N/A	34 (65)	26 (72)	22 (63)
RAS inhibitor use[c], *n* (%)	N/A	32 (62)	(56)	(63)

Distribution of hyperuricaemic cases is as follows: Study-B, 5 randomised to placebo and 4 to exenatide; Study-C, 2 randomised to placebo and 4 to liraglutide; Study-D, 6 randomised to insulin glulisine and 1 to lixisenatide. [a]Not calculated because of interference with insulin glargine. [b]Defined as plasma UA >6.8 mg/dL.[6] [c]Use of losartan which has an uricosuric effect, was as follows: Study-B, 1 randomised to placebo and 1 to exenatide; Study-C, 1 randomised to placebo and 1 to liraglutide; Study-D, 1 randomised to insulin glulisine and 2 to lixisenatide.

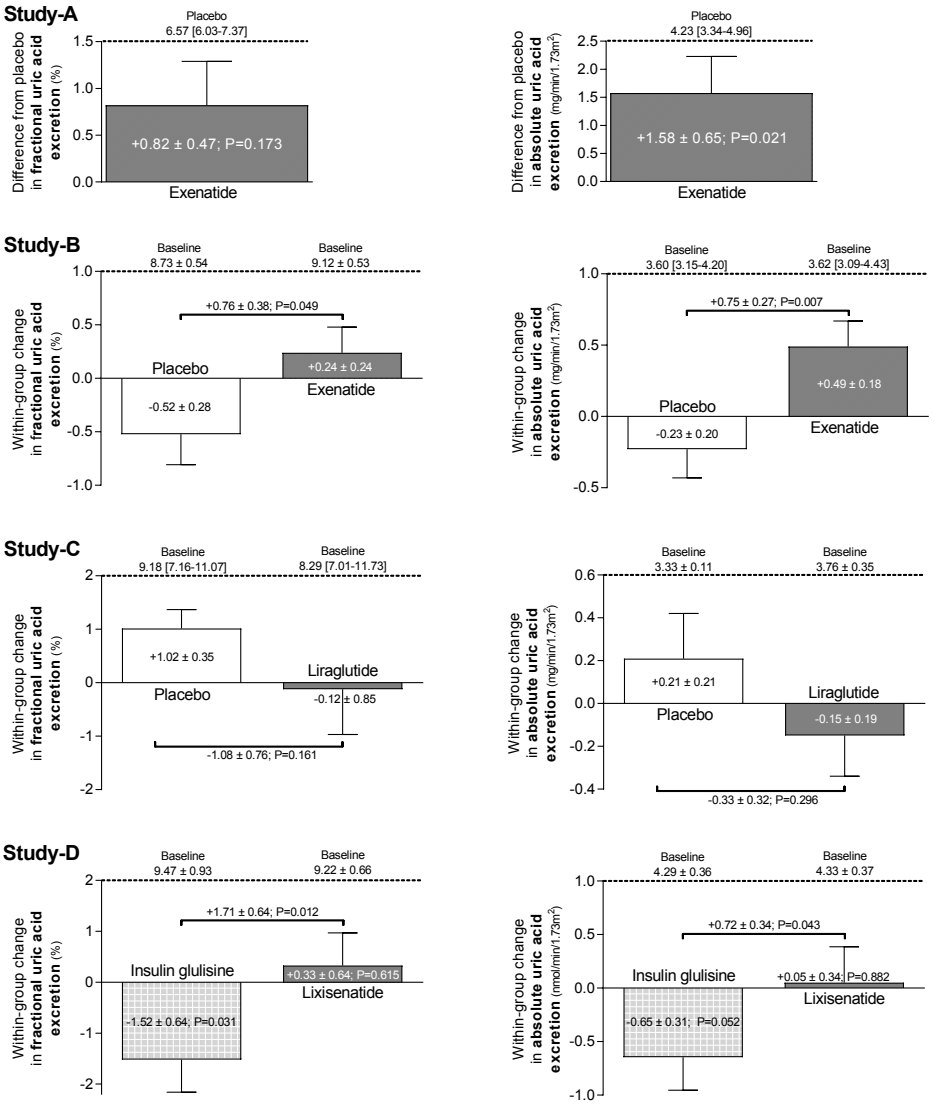

Figure 2. Effect of immediate and prolonged GLP-1RA administration in healthy overweight males (Study-A) and T2DM patients (Study-B–Study-D). Data are given as mean ± SEM, median [IQR], or baseline-corrected mean difference ± SEM. Wilcoxon signed-rank test was used to examine effects of exenatide vs placebo in Study-A. Multiple linear regression, corrected for correponding baseline values, was used to determine exenatide-induced effects compared to placebo in Study-B, liraglutide-induced effects compared to placebo in Study-C and lixisenatide-induced effects compared to insulin glulisine in Study-D. Paired t-test was used for within-group comparisons in Study-D

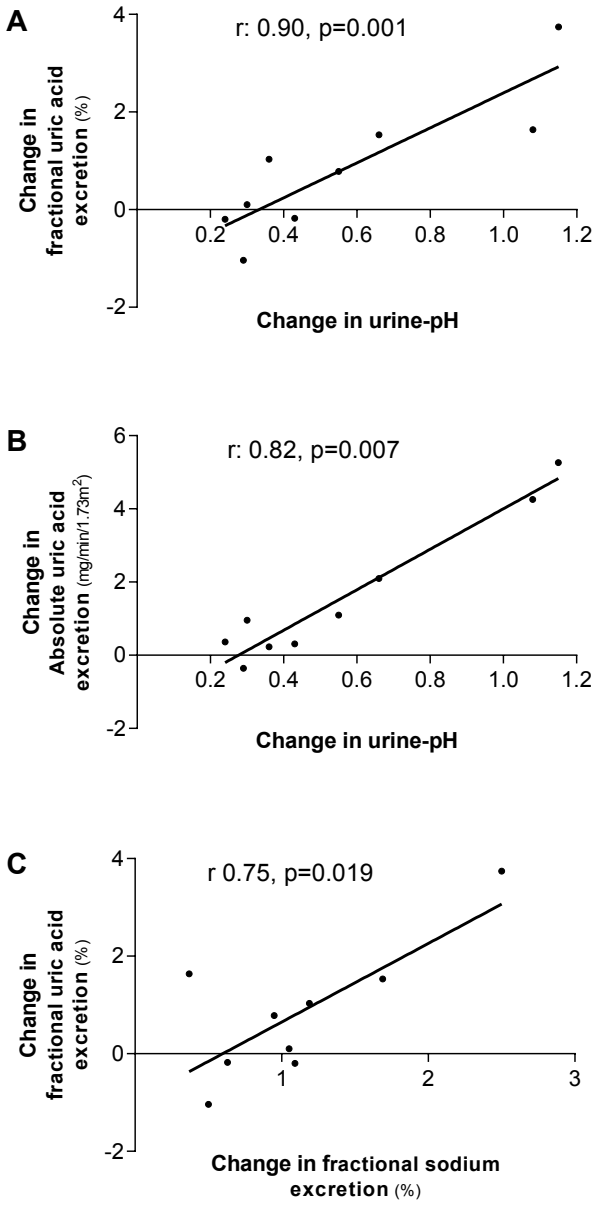

Figure 3. Correlations of within-group changes in healthy overweight men (Study-A). A and B, Spearman signed rank test or C, Pearson's correlation was used to assess correlations

(Figure 5), while fractional UE_{UA} or absolute UE_{UA} did not correlate with baseline plasma UA (both p>0.5) or with changes in plasma UA (both p>0.06). No correlations were observed in the liraglutide arm or the placebo arm between changes from baseline to Week 2 in creatinine-based fractional UE_{UA} and baseline plasma UA (p>0.6).

Study-D

The study population comprised 35 patients (Table 1). At baseline, mean ± SD fasting UA was 5.68 ± 1.19 mg/dL (7 patients were hyperuricaemic), which decreased after the meal by 0.09 ± 0.04 mg/dL (p=0.03). Prior to randomisation, creatinine-based fractional UE_{UA} tended to increase slightly after the meal by 0.02 ± 0.01% (p=0.051). After 8 weeks of treatment, fasting and postprandial UA did not differ between or within treatment arms (all p>0.1). Within groups, lixisenatide treatment did not affect postprandial fractional UE_{UA} (p=0.6) or absolute UE_{UA} (p=0.9), while insulin glulisine reduced fractional UE_{UA} (−1.52 ± 0.64; p=0.03) and tended to reduce absolute UE_{UA} (−0.65 mg/min/1.73 m^2; p=0.052). This resulted in significant between-group differences (Figure 2). As previously reported, urine pH and fractional UE_{Na}increased with lixisenatide compared to insulin glulisine in the postprandial state, while GFR was not

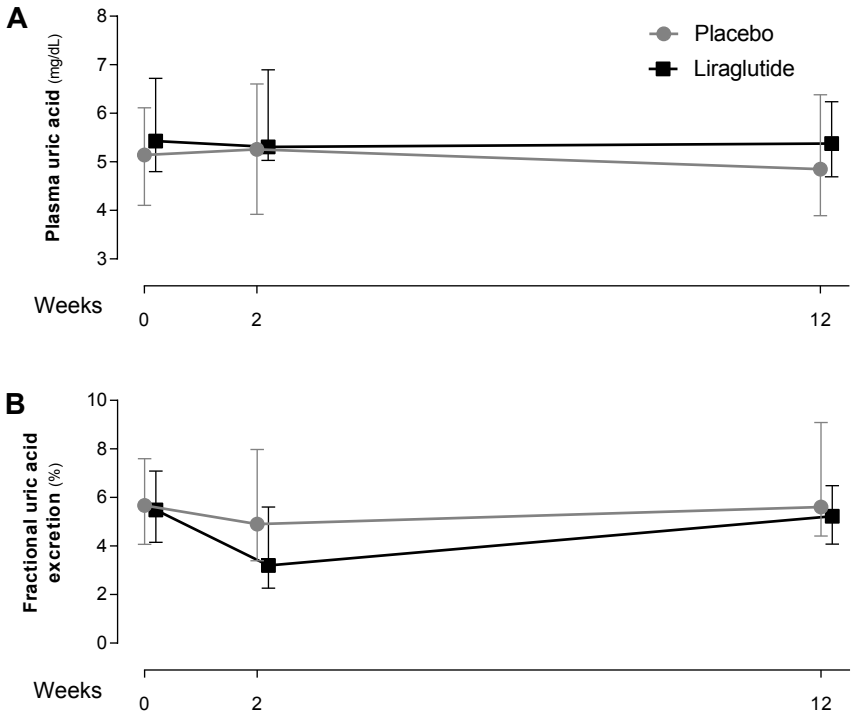

Figure 4. Effect of 12-week treatment with liraglutide in T2DM patients on A, creatinine-based fractional uric acid excretion and B, plasma uric acid. Data are given as median [IQR]. Multiple linear regression corrected for corresponding baseline values was used to examine liraglutide-induced effects compared to placebo. No significant differences were found

affected (Supplemental Table 4).[23] Postprandial urinary glucose did not differ between (p=0.2) or within groups (both p>0.6). Compared to insulin glulisine, lixisenatide did not change HbA_{1c} (p=0.9), but decreased blood glucose during kidney tests by 1.8 ± 0.5 mmol/L (p=0.002). Within groups, insulin concentrations decreased with lixisenatide (Supplemental Table 4).[23] Body weight decreased by 1.4 ± 0.6 kg with lixisenatide compared to insulin glulisine (p=0.03).[23] No within- or between-group differences in postprandial PRC or angiotensin II were observed.[23] In the lixisenatide and insulin glulisine arms, changes from baseline to Week 8 in plasma UA correlated with fasting baseline plasma UA (r: –0.52, p=0.031 and r: –0.52, p=0.041, respectively) (Figure 5B). Fasting or postprandial fractional UE_{UA} did not correlate with baseline plasma UA (all p>0.08) or with changes in plasma UA (all p>0.3). Changes in absolute UE_{UA} correlated with baseline plasma UA in the lixisenatide arm (r: –0.51, p=0.038) (Figure 5C), but not in the insulin glulisine arm (r: 0.30, p=0.3), while changes in absolute UE_{UA} did not correlate with changes in plasma UA in either treatment group (both p>0.3).

Discussion

We are the first to determine the immediate and chronic effects of different GLP-1RAs on UA levels and kidney UA clearance in humans using *post-hoc* analyses of 4 controlled clinical intervention trials. We demonstrate that GLP-1RA exenatide infusion immediately increases UE_{UA} in healthy overweight males and T2DM patients. Plasma UA levels increased slightly in healthy subjects, but were unaffected in the T2DM population. In contrast, prolonged treatment with 2 pharmacologically different GLP-1RAs (ie, 12 weeks of liraglutide [long-acting agent] or 8 weeks of lixisenatide [short-acting agent]) did not affect UE_{UA} or plasma UA-levels in T2DM patients (of whom ~20% fulfilled the criteria of hyperuricaemia).

Recent landmark cardiovascular safety trials indicate that GLP-1RAs provide cardiovascular and renal benefit compared to placebo (ie, standard of care) in at-risk T2DM patients after prolonged therapy.[13-15,32] However, underlying cardio-renal protective mechanisms remain incompletely understood. Hyperuricaemia is frequently observed in patients with pre- or recent-onset T2DM,[33] and increased levels of UA are thought to mediate, in part, cardiovascular and renal risk in T2DM.[4,8] As GLP-1-peptide administration for 10 weeks in diabetic rats reduces UA levels,[20] and GLP-1RAs exhibit clinically relevant tubular effects in humans,[21-24] we hypothesised that GLP-1RA administration lowers plasma UA by increasing kidney UA clearance.[25-27] Although intravenous exenatide administration rapidly increased UE_{UA} in overweight healthy subjects (Study-A) and T2DM patients (Study-B), no reduction in UA-levels was observed. This could be explained by the short duration of the studies, although we cannot exclude that exenatide concurrently increased UA production. Furthermore, upon prolonged treatment with either a short-acting or long-acting GLP-1RA, we did not observe effects on UA clearance or UA levels. As such, our results indicate that it is unlikely that UA is a relevant clinical mediator of the recently described cardio-renal benefits of GLP-1RAs in T2DM patients.

Although the tubular handling of UA in humans is incompletely understood, we propose several mechanisms by which exenatide infusion could have immediately increased UE_{UA} in

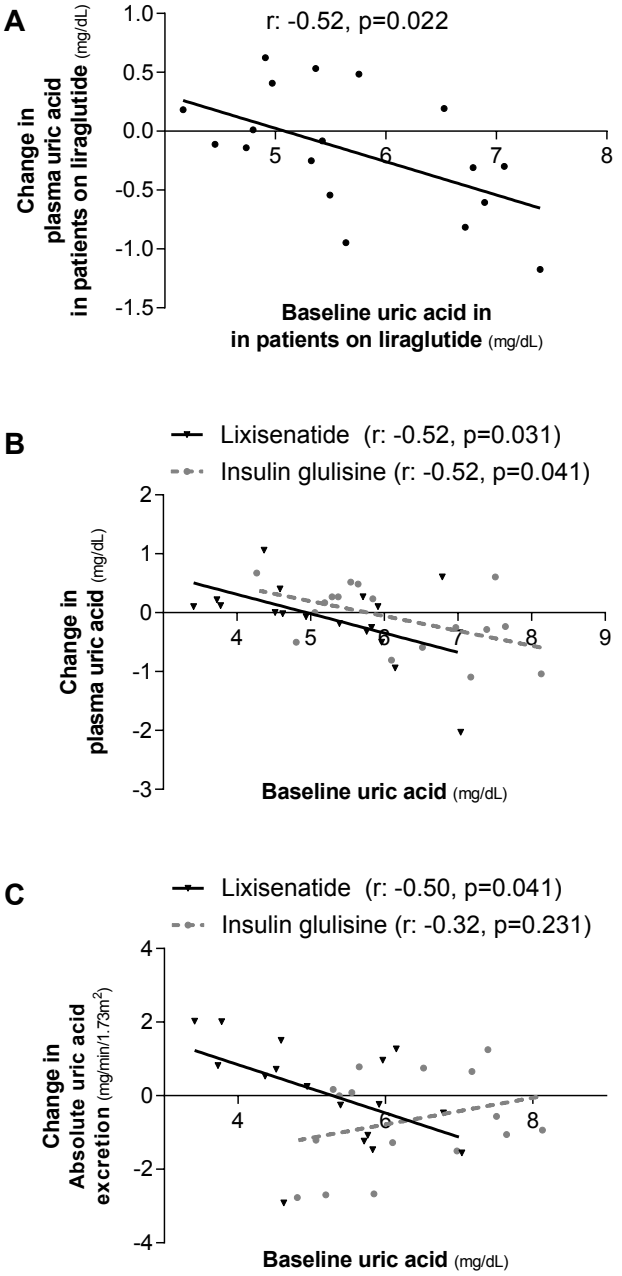

Figure 5. Correlations in T2DM patients receiving A, liraglutide (Study-C) or B and C, lixisenatide or insulin glulisine (Study-D). Spearman signed rank test was used to assess correlations

our studied populations, based on correlation and regression analyses. The effect seems to be driven, to large extent, by a concurrent increase in urine pH and, in part, by an increase in UE_{Na}. Inhibition of NHE3 by GLP-1RA[21-24] may have decreased pH-dependent organic acid

transporter (OAT)-4 activity,[25] thereby reducing kidney UA reabsorption. In addition, urinary alkalization[26,27] or increased UE_{Na} [2,34] may have contributed directly to the uricosuric effect of acute exenatide administration. While high concentrations of urinary glucose may stimulate UE_{UA} via the tubular GLUT9 transporter,[2,35,36] we did not observe any relevant glycosuria in our T2DM patient population at baseline or after treatment, which makes such a pathway unlikely. Furthermore, statistical adjustment for the observed decrease in blood glucose, which could result in modest reductions in glycosuria and consequent UA excretion, did not increase UE_{UA}. We also suggest that the exenatide-induced increase in insulin blunted the increase in UE_{UA}, because insulin is known to augment tubular UA-reabsorption.[2,35] Finally, GLP-1RA can decrease renin-angiotensin-aldosterone system (RAAS) activity,[37-39] which could increase UE_{UA} by lowering angiotensin-II.[2] However, as we and others[40,41] did not observe changes in PRC or angiotensin-II, there seems to be no important role for the circulating RAAS, although a contribution of the intrarenal renin-angiotensin system cannot be excluded.

In order to explore the sustainability of the immediate GLP-1RA-induced effect on UE_{UE}, and to determine whether this translates into lowering of plasma UA upon long-term treatment, we analysed the data of 2 clinical intervention trials involving structurally different GLP-1RAs (Study-C and Study-D). GLP-1RAs can be categorized as either short-acting/prandial compounds, which provide short-lived receptor activation (eg, exenatide and lixisenatide) or as long-acting compounds (eg, exenatide once-weekly and liraglutide), which activate GLP-1 receptors continuously. The pharmacokinetic differences between these drugs are known to lead to clinically relevant differences in their pharmacodynamic profiles. For example, because of their continuous receptor stimulation and resultant tachyphylaxis, the ability of long-acting GLP-1RAs to decrease the gastric emptying rate and to inhibit areas in the central nervous system involved in hedonic feeding are not sustained during prolonged treatment.[29,30] In line with these observations, we recently suggested that tachyphylaxis and/or induction of compensatory mechanisms at the tubular level explains that only short-acting GLP-1RAs harbour sustained natriuretic and urine-alkalising actions during prolonged treatment.[23,28] We hypothesised that such divergent actions could also emerge with respect to tubular UA handling. Indeed, 12 weeks of treatment with the long-acting GLP-1RA liraglutide compared to placebo, did not affect UE_{UA}. Interestingly, although 8 weeks of treatment with the short-acting GLP-1RA lixisenatide increased $UE_{UA,}$ compared to insulin glulisine, this was entirely mediated by an anti-uricosuric effect in the insulin glulisine arm. Thus, these results do not indicate that lixisenatide affects UE_{UA}. Finally, UA levels were unaffected by either GLP-1RA in our prolonged intervention studies, despite decreases in body weight, thus potentially improving insulin resistance.[42]

In comparison, the initial stimulating effects of an SGLT2 inhibitor on UE_{UA} also seem to wane upon prolonged intervention, as suggested by a mechanistic study in healthy subjects and by in vitro transport experiments.[43] However, in contrast to current GLP-1RA data, chronic SGLT2 inhibitor therapy does have a sustained suppressive effect on plasma-UA,[36] suggesting that these drugs establish a new steady state in UA metabolism and clearance.

Notably, our correlative analyses suggest that prolonged GLP-1RA treatment may lower plasma UA in T2DM patients with higher baseline plasma UA levels. However, as a similar

relation is observed in patients treated with insulin glulisine, a glucose-dependent mechanism is suggested.[35] Accordingly, individual responses in renal UA handling were not associated with changes in plasma UA, although the isolated correlation of changes in absolute UE_{UA} with plasma UA in patients treated with lixisenatide merits further research.

The described studies are not without limitations. First, intestinal UA excretion and UA formation were not assessed. Such measurements are technically challenging, and intestinal excretion was not expected to be affected substantially.[3] UA formation could theoretically be influenced by treatment through alterations in circulating fructose levels.[35] We did not assess fructose levels in these trials, but emphasize that this would be confounded by glucose lowering. Second, we did not measure UA crystallization as the increment in urine pH dramatically improves UA solubility, especially with pH > 6.0 to 6.5,[44] rendering the likelihood of precipitation negligible. It is tempting to speculate that this would potentially also prevent UA-mediated tubulopathy.[35] However, we did not observe any effects on glomerular and tubular injury markers (albumin-creatinine ratio, KIM-1 or NGAL) as previously reported in T2DM patients without overt nephropathy.[23,24,28] Third, since the intervention conditions in Study-A were performed sequentially, we cannot exclude time-dependent effects. This may explain the somewhat unexpected slight increase in plasma UA after immediate exenatide infusion in healthy subjects. Fourth, our T2DM population had a prolonged history of diabetes and we did not specifically include patients with (symptomatic or asymptomatic) hyperuricaemia, which does not allow generalisation of the current data to patients with a shorter history of diabetes and/or higher UA levels at baseline.[33,45] Fifth, T2DM patients in Study-B and Study-C had relatively low cardiovascular risk, and the duration of follow-up in Study-C and Study-D was relatively short, both of which may limit direct generalisation to the recently published GLP-1RA cardiovascular outcome trials. Interestingly, the association between UA levels and progression of diabetic kidney disease appears to be present only in healthier subjects, that is, those with earlier stages of chronic kidney disease (CKD),[46] and seems to be lost in more advanced CKD.[47,48] Sixth, the effects of lixisenatide were investigated in the postprandial state and an active comparator was used rather than placebo, which may have led to a more heterogeneous response and thus hampers direct comparison with other reported studies that were performed in the fasting state. Seventh, lixisenatide markedly slows the gastric emptying rate,[23] which could affect purine uptake in the postprandial state. However, the effects of the mixed breakfast on plasma UA and UE_{UA} were minor at baseline. Finally, although we did not test prolonged treatment effects of exenatide twice-daily, the drug is structurally similar to lixisenatide, and harbours comparable pharmacokinetic and pharmacodynamic properties.[49] As such, we believe it is unlikely that prolonged treatment with this formulation of exenatide would result in differential effects on kidney UA clearance and/or UA levels.

In conclusion, immediate exenatide infusion increases UE_{UA} in healthy overweight men and in T2DM patients by increasing UE_{UA}, possibly mediated by GLP-1RA-induced NHE3 inhibition in the proximal tubule and/or consequent urine alkalization. Prolonged treatment with the long-acting GLP-1RA liraglutide or the short-acting GLP-1RA lixisenatide does not affect UE_{UA} or UA levels in T2DM patients with normal plasma UA levels and relatively low

cardiovascular risk. Our results indicate that it is unlikely that UA is a relevant mediator of the cardiovascular and renal benefits of GLP-1RA.

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Supplemental methods

Participants

Participants of all studies were recruited by advertisements in local newspapers. Detailed descriptions of all inclusion and exclusion criteria were reported previously.[1-5] In brief, Study-A[1] included 10 healthy, overweight (body mass index [BMI]>25 kg/m^2), Caucasian males, aged 18-30 years. Study-B[4] and Study-C[5] included 60 insulin naive T2DM patients (HbA_{1c} 6.5%-9.0% [48-75 mmol/mol]) with a BMI ranging from 25 to 40 kg/m^2. All were Caucasian, men or post-menopausal women and aged 35-75 years, who were treated with a stable dose of metformin and/or sulphonylurea for ≥ 3months. Study-D[3] included 40 T2DM patients (HbA_{1c} 6.5-10.0% [48-86 mmol/mol]) with a BMI >25 kg/m^2. All were Caucasian, men or post-menopausal women, aged 35-75 years, who were treated with a stable dose of insulin glargine (± 20% for ≥ 3 months), with or without a stable dose of metformin for ≥ 3 months. Key exclusion criteria for T2DM patients in all studies included history of pancreatic or active liver disease, malignancy, estimated (GFR) <60 mL/min/1.73 m^2, current urinary tract infection or nephritis, and use of diuretics that could not be stopped 3 months prior to and during the intervention period. Complete bladder emptying in all participants was ascertained by bladder ultrasonography at the screening visit.

Interventions and randomisation

In Study-A and Study-B, subjects received intravenous administration with either exenatide 10 μg (AstraZeneca, London, UK) or placebo (equivalent volume of isotonic saline), dissolved in 46 mL of isotonic saline and 4 mL of the participants blood. The infusion schedule was shown to yield plasma exenatide levels within the therapeutic range (130-150 pg/ml).[6,7]

In Study-C, patients received prefilled pens for subcutaneous injection containing visually identical liraglutide (Novo Nordisk A/S, Bagsværd, Denmark) or placebo, to be taken once-daily in the evening for 12 weeks. A dose-increment schedule was used (Week-1: 0.6 mg; Week-2: 1.2 mg; Weeks 3 to 12: 1.8 mg), and depending on drug tolerance, time between dose-increments could be extended or the drug could be decreased at the investigator's discretion. Study-C also included a third treatment-arm with the oral dipeptidyl peptidase -4 inhibitor sitagliptin 100 mg once-daily; given the double-dummy design, all patients were using oral capsules in addition to their subcutaneous injections.[5] Here we report the results of the liraglutide and placebo treatment-arms only.

In Study-D, patients received lixisenatide (Sanofi Aventis, Paris, France) or insulin glulisine (Sanofi Aventis, Frankfurt am Main, Germany). Lixisenatide was administered 10 μg once-daily for 2 weeks, followed by a maintenance dose of 20 μg once-daily for the remainder of the study, injected subcutaneously ~30 minutes before breakfast. Patients with an HbA_{1c} between 6.5-7.5% at screening initially lowered their insulin glargine dose by 20% to limit the risk for hypoglycaemia as previously described.[3] Insulin glulisine was initiated with a dose of 2 units/day once-daily, injected <15 minutes before breakfast, and was self-titrated to a 2-hour postprandial glucose target between ≥5.0 and ≤8.0 mmol/L.

Randomisation was performed by the trial pharmacist using computer generated lists, with an allocation ratio of 1:1 (Study-B and Study-D) or 1:1:1 (Study-C), and a block size of four (Study-D) or six (Study-B and Study-C).[4,5]

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Supplemental data

Supplemental Table 1. Kidney and metabolic responses to GLP-1 receptor agonist exenatide-infusion in healthy overweight males (*Study-A*)[1]

	(N=9)		
	Placebo	**Exenatide**	**P-value**
Kidney variables			
GFR (mL/min/1.73m^2)	97 ± 12	114 ± 16	**0.021**
FE_{Na} (%)	1.34 ± 0.46	2.45 ± 0.91	**0.001**
Abs_{Na} (µmol/min/1.73m^2)	172 ± 72	379 ± 160	**0.001**
Urine-osmolality (mOsm/kg)	163 ± 35	443 ± 98	**0.005**
Urine-pH	6.53 [6.03–6.90]	7.04 [6.89–7.34]	**<0.001**
Metabolic variables			
Glucose (mmol/L)	4.31 [4.20–4.43]	3.77 [3.63–3.91]	**0.005**
Insulin (pmol/L)	35.2 ± 16.6	32.4 ± 12.4	0.500

Data are means ± SEM or median [IQR]. p-values are based on paired t-test or Wilcoxon signed rank test. p<0.05 is shown in bold font. Abs, absolute excretion; FE, fractional excretion; GFR, glomerular filtration rate.

Supplemental Table 2. Kidney and metabolic responses to placebo - or GLP-1 receptor agonist exenatide-infusion in overweight patients with type 2 diabetes (*Study-B*)[4]

	Placebo (N=28)		Exenatide (N =24)		
	Baseline	**Intervention**	**Baseline**	**Intervention**	**P-value**
Kidney variables					
GFR (mL/min/1.73m^2)	82 ± 4	83 ± 3	83 ± 3	86 ± 4	0.489
FE_{Na} (%)	1.24 ± 0.11	1.33 ± 0.10	1.22 ±0.10	1.61 ± 0.13	**<0.001**
Abs_{Na} (µmol/min/1.73m^2)	127 ± 10	153 ± 10	134 ± 10	189 ± 13	**0.008**
Urine-osmolality (mOsm/kg)	204 ± 32	225 ± 14	188 ± 15	355 ± 24	**<0.001**
Urine-pH	5.76 ± 0.11	5.71 ± 0.11	5.91 ± 0.11	6.53 ± 0.12	**<0.001**
Metabolic variables					
Glucose (mmol/L)	6.6 ± 0.2	6.0 ± 0.2	6.5 ± 0.2	4.5 ± 0.2	**<0.001**
Insulin (pmol/L)	102 ± 12	70 ± 8	89 ± 11	88 ± 10	**<0.001**

Data are means ± SEM or median [IQR]. *P*-values are based on multivariable linear regression to examine exenatide-induced effects versus placebo, and corrected for potential between-group baseline differences. p<0.05 is shown in bold font. Abs, absolute excretion; CR, creatinine ratio; FE, fractional excretion; GFR, glomerular filtration rate.

Supplemental Table 3. Kidney and metabolic responses to placebo or GLP-1 receptor agonist liraglutide treatment for 12 weeks in overweight patients with type 2 diabetes (*Study-C*) [5]

	Placebo (N=17)		Liraglutide (N=19)		
	Week-0	**Week-12**	**Week-0**	**Week-12**	**P-value**
Kidney variables					
GFR (mL/min/1.73m^2)	83 ± 4	85 ± 4	79 ± 3	84 ± 4	0.169
FE_{Na} (%)	1.30 [0.91-1.34]	1.34 [0.66-1.97]	1.14 [0.95-1.41]	1.04 [0.62-1.37]	0.162
Abs_{Na} (µmol/min/1.73m^2)	121 ± 11	142 ± 22	137 ± 9	138 ± 18	0.512
Urine-osmolality (mOsm/kg)	170 [121-241]	138 [114-182]	154 [117-221]	139 [91-153]	0.106
Urine-pH	5.64 ± 0.14	5.61 ± 0.15	5.98 ± 0.12	5.52 ± 0.14	0.341
Metabolic variables					
HbA_{1c} (%)	7.5 ± 0.2	8.0 ± 0.3	7.4 ± 0.2	6.6 ± 0.2	**<0.001**
HbA_{1c} (mmol/mol)	58 ± 2	64 ± 4	57 ± 2	57 ± 2	**<0.001**
Glucose (mmol/L)	8.9 ± 0.5	9.4 ± 0.5	8.3 ± 0.3	7.3 ± 0.4	**<0.001**
Insulin (pmol/L)	106 ± 17	88 ±13	92 ±14	125 ± 20	**0.020**

Data are means ± SEM or median [IQR]. *P*-values are based on multivariable linear regression to examine liraglutide-induced effects versus placebo, and corrected for potential between-group baseline differences. p<0.05 is shown in bold font. Abs, absolute excretion; CR, creatinine ratio; FE, fractional excretion; GFR, glomerular filtration rate; HbA_{1c}, glycated haemoglobin

Supplemental Table 4. Kidney and metabolic responses to titrated insulin glulisine or GLP-1 receptor agonist lixisenatide treatment for 8 weeks in overweight patients with type 2 diabetes (*Study-D*) [3]

	Insulin glulisine (N=18)		Within-group P-value	Lixisenatide (N=17)		Within-group P-value	Between-group P-value
	Week-0	Week-8		Week-0	Week-8		
Kidney variables							
GFR (mL/min/1.73m^2)	81 ± 4	83 ± 5	0.589	93 ± 3	92 ± 4	0.912	0.989
Inulin-based FE_{Na} (%)	0.96 ± 0.09	0.93 ± 0.07	0.768	0.81 ± 0.04	0.96 ± 0.07	**0.021**	0.214
$_{\Delta PP\text{-}F}$Creatinine-based FE_{Na} (%)	0.03 ± 0.07	-0.11 ± 0.08	**0.047**	0.08 ± 0.04	0.18 ± 0.05	**0.043**	**0.003**
Abs_{Na} (µmol/min/1.73m^2)	108 ± 13	108 ± 8	0.975	105 ± 6	124 ± 10	0.051	0.114
Urine-osmolality (mOsm/kg)	225 [182-375]	298 [244-393]	0.137	304 [262-512]	293 [245-349]	0.094	0.216
Urine-pH	5.24 ± 0.14	5.12 ± 0.09	0.222	5.09 ± 0.09	5.48 ± 0.18	**0.024**	**0.013**
Metabolic variables							
HbA_{1c} (%)	7.8 ± 0.2	7.2 ± 0.1	**0.001**	8.3 ± 0.2	7.5 ± 0.2	**<0.001**	0.897
HbA_{1c} (mmol/mol)	62 ± 2	55 ± 2	**<0.001**	67 ± 2	59 ± 2	**<0.001**	0.961
Glucose (mmol/L)	10.1 [8.7-11.7]	8.0 [7.0-8.9]	**<0.001**	10.2 [8.9-11.4]	5.7 [5.3-6.7]	**<0.001**	**0.002**
Insulin (pmol/L)	412 [314-769]	388 [310-710]	0.341	333 [232-475]	260 [198-350]	**0.001**	0.057

Data are means ± SEM or median [IQR]. p-values are based on multivariable linear regression to examine liraglutide-induced effects versus placebo, and corrected for potential between-group baseline differences. P<0.05 is shown in bold font. Endogenous insulin plus insulin glargine concentrations are determined by the insulin assay. The insulin assay does not measure insulin glulisine. Abs, absolute excretion; CR, creatinine ratio; FE, fractional excretion; F, fasting; GFR, glomerular filtration rate; HbA_{1c}, glycated haemoglobin; PP, postprandial.