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Acute renal haemodynamic effects of glucagon-like peptide-1 receptor agonist exenatide in healthy overweight men



Marcel H. A. Muskiet
Lennart Tonneijck
Mark M. Smits
Mark H. H. Kramer
Michaela Diamant
Jaap A. Joles
Daniël H. van Raalte

Abstract

Aims

To determine the acute effect of glucagon-like peptide-1 (GLP-1) receptor agonist exenatide and the involvement of nitric oxide (NO) on renal haemodynamics and tubular function, in healthy overweight men.

Methods

Renal haemodynamics and tubular electrolyte handling were measured in 10 healthy overweight men (aged 20–27 years; BMI 26–31 kg/m²) during intravenous administration of placebo (saline 0.9%), exenatide, and exenatide combined with the NO-synthase inhibitor L-N^G-monomethyl arginine (L-NMMA). Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were determined by inulin and para-aminohippurate clearance techniques, respectively, based on timed urine sampling. Glomerular hydrostatic pressure and vascular resistance of afferent and efferent renal arterioles were calculated using the Gomez formulae. Urinary electrolytes, osmolality and pH were also measured.

Results

GFR increased by a mean of 18 ± 20 (+20%; $p=0.021$) and ERPF increased by a median (interquartile range) of 68 (26; 197) mL/min/1.73 m² (+14%; $p=0.015$) during exenatide infusion versus placebo. During L-NMMA infusion, exenatide increased GFR by mean 8 ± 12 mL/min/1.73 m² (+9%; $p=0.049$). Exenatide increased estimated glomerular pressure by +6% ($p=0.015$) and reduced afferent renal vascular resistance by –33% ($p=0.038$), whereas these effects were blunted during L-NMMA infusion. Exenatide increased absolute and fractional sodium excretion, urinary osmolality and urinary pH. The tubular effects of exenatide were not altered by concomitant L-NMMA infusion.

Conclusions

Exenatide infusion in healthy overweight men acutely increases GFR, ERPF and glomerular pressure, probably by reducing afferent renal vascular resistance, and at least partially in an NO-dependent manner. As baseline renal haemodynamics in patients with type 2 diabetes differ from those in healthy individuals, clinical studies on the renal effects of GLP-1 receptor agonists are warranted.

Introduction

Glucagon-like peptide-1 (GLP-1) receptor agonists are injectable antihyperglycaemic drugs that are widely used in the treatment of type 2 diabetes (T2DM). GLP-1 receptor agonists improve glycaemia by enhancing insulin and suppressing glucagon secretion in a glucose-dependent manner, thereby carrying a low hypoglycaemia risk.¹ In addition to their pancreatic effects, mounting evidence indicates that GLP-1 receptor agonists also exert actions on other organ systems, including the kidneys.² Although sporadic cases of acute renal failure were reported shortly after drug approval,³ GLP-1 receptor agonists have now been suggested to exhibit glucose-independent renoprotective properties, which may prevent the onset and progression of diabetic kidney disease in patients with T2DM.⁴ Indeed, GLP-1 receptor agonists have been shown to reduce the surrogate renal endpoint albuminuria in both phase III trials² and in the recent cardiovascular safety outcome study of GLP-1 receptor agonist lixisenatide in patients with T2DM.⁵ Their effects on established hard renal endpoints, however, are still to be determined.

The mechanisms by which GLP-1 receptor agonists influence the renal system are unclear, but may involve effects on renal haemodynamics [glomerular filtration rate (GFR) and effective renal plasma flow (ERPF)]. Interestingly, 3-h GLP-1 peptide infusion attenuated the supraphysiologically increased GFR (glomerular hyperfiltration) in obese insulin-resistant males, as measured by creatinine clearance.⁶ As glomerular hyperfiltration is assumed to reflect increased glomerular hydrostatic pressure (P_{GLO}), leading to stress-induced glomerular injury, GLP-1-induced reductions in GFR are regarded as clinically beneficial;⁴ however, contrasting results have been reported. In healthy rats, GLP-1 increased GFR and renal blood flow (RBF),⁷⁻¹⁰ whereas studies in healthy lean males with normal GFR showed no significant effects of GLP-1 infusion on GFR and ERPF.^{6,11,12}

Hitherto, no studies have examined the actions of clinically used GLP-1 receptor agonists on human renal physiology. The first and commonly used GLP-1 receptor agonists exenatide mimics many, but not all, effects of native GLP-1.^{13,14} The aim of the present study, therefore, was to investigate the acute effects of exenatide on renal haemodynamic functions in healthy overweight men using classic clearance methodology. As exenatide may influence GFR and renal arteriolar tone through interactions with (local) neurohormonal/vascular factors [such as nitric oxide (NO)], measurements were also performed during concomitant infusion of the non-selective NO synthase inhibitor L-N^G-monomethyl-arginine (L-NMMA).

Materials and methods

Participants

Ten healthy overweight (body mass index $>25 \text{ kg/m}^2$) Caucasian male volunteers, aged 18–30 years, were enrolled after local advertisements (Table 1). Normal health was ascertained during a screening visit,¹⁵ during which medical history, physical examination, 2-h 75-mg oral glucose tolerance test and blood/urine analyses did not reveal any abnormalities. Subjects were not allowed to use any medication. An ultrasonic bladder scan was performed to ensure total

bladder emptying. The study was approved by the local ethics review board, and conducted in accordance with the Declaration of Helsinki and International Conference on Harmonisation on Good Clinical Practice. All subjects provided written informed consent before participation. The study was registered at <https://clinicaltrials.gov> (ID: NCT01744236).

Study design

This was a single-centre, non-blinded, placebo-controlled intervention study performed over two separate testing days, which were planned in no particular order and separated by at least 2 days to prevent carry-over effects. During the first visit, the acute effects of placebo (isotonic 0.9% saline) and subsequent exenatide infusion on renal physiology were evaluated. On the second visit, the same experimental scheme was performed during concomitant intravenous (i.v.) administration of L-NMMA (Figure 1).

Two days before the study visits, the subjects were instructed to adhere to a controlled sodium chloride (9–12 g/day) and protein (1.5–2.0 mg/kg/day) diet, to reduce diet-induced variation in renal physiology. In addition, before the experiments, the subjects refrained from heavy physical activity and intake of alcohol for >24 h, and did not consume caffeine for >12 h. After an overnight fast, participants drank 500 ml of water (to stimulate diuresis) before arriving at the clinical research unit at 07:30 hours. Subjects remained in a semi-recumbent position in a temperature-controlled room ($23.0 \pm 1.0^\circ\text{C}$) throughout the testing day. I.v. catheters were placed in an antecubital vein of both forearms to allow intermittent blood sampling on the non-dominant side, and continuous infusion of the renal tracer substances and study drugs on the other.

Diuresis was maintained by oral intake of 10 ml/kg (maximum 1000 ml) tap water during the 90-min inulin/PAH equilibration period, followed by 200 ml/h of tap water for the remainder of the testing day. A controlled amount (2 ml) of isotonic 0.9% saline was used to flush i.v. lines after blood sampling, and an average isotonic 0.9% saline infusion rate of 10 ml/h was sustained throughout the testing procedures.

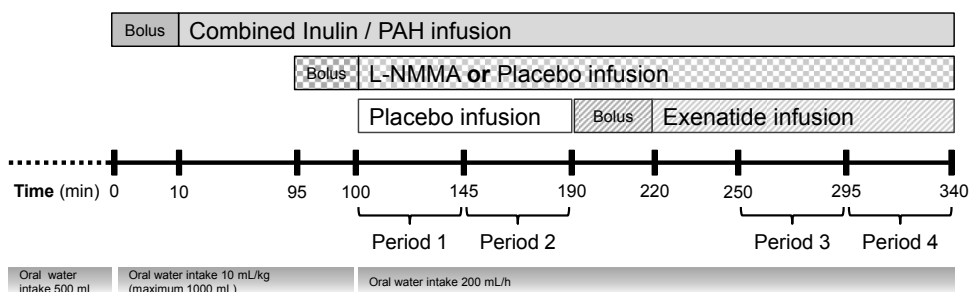


Figure 1. Outline of experimental procedure. L-NMMA, L-N^G-monomethyl arginine.

Renal haemodynamics

The GFR and ERPF values were determined using a standard method renal clearance technique based on timed urine sampling using inulin (Inutest®; Fresenius-Kabi Austria GmbH, Graz, Austria) and PAH (aminohippurate sodium 'PAH' 20%; Merck Sharp & Dohme International, Whitehouse Station, NJ, USA), respectively. After 60–75 min acclimatization, blood was collected for determination of blank serum inulin and PAH concentrations, after which a 10-min priming infusion of inulin (45 mg/kg body weight) and PAH (6 mg/kg body weight) was administered. Subsequently, inulin and PAH were infused continuously at a controlled rate of 22.5 and 11 mg/min, respectively. After a 90-min equilibration period, urine was collected by spontaneous voiding every 45 min for two periods per intervention condition (Figure 1). Subjects were allowed to stand upright during voiding, and were requested to urinate until a subjective feeling of complete bladder emptying was reached. Urine samples were analysed for inulin, PAH, electrolytes, osmolality and pH. Before and after each collection period, blood samples were analysed for inulin, PAH and electrolytes. Haematocrit was determined between the two collection periods. All presented renal clearance and tubular function data are averaged data of the two consecutive independent 45-min urine collection periods per intervention condition. All renal haemodynamic variables were adjusted for body surface area, calculated using the Mosteller formula.¹⁶ The single frequency bioelectrical impedance analyser Maltron-BF-906 (Maltron International Ltd, Rayleigh, UK) was used to assess body water percentage between the two urine collection periods.

Calculation of intrarenal haemodynamic and tubular functions

The RBF was calculated by the formula: ERPF/(1–haematocrit). Filtration fraction was derived using GFR/ERPF, and renal vascular resistance using mean arterial pressure (MAP)/RBF. Fractional excretion (FE) was calculated as follows:

$$FE_{[\text{electrolyte}]} = \frac{([\text{electrolyte}]_{\text{urine}} \times \text{inulin}_{\text{plasma}})}{([\text{electrolyte}]_{\text{plasma}} \times \text{inulin}_{\text{urine}})}$$

Intrarenal haemodynamics [including P_{GLO} and resistance of afferent (R_A) and efferent (R_E) renal arterioles] were calculated according to the model originally established by Gomez (Supplemental methods).¹⁷

Systemic haemodynamics

Systolic and diastolic blood pressure (BP), MAP and heart rate were measured repeatedly using an automated oscillometric device (Dinamap®; GE Healthcare, Little Chalfont, UK) over the brachial artery of the non-dominant arm. Measurements were performed in triplicate at 1–2-min intervals; the mean of the last two measurements was taken as final value.

Study drugs

A dose of exenatide 10 µg (AstraZeneca, London, UK) was diluted in 46 ml isotonic 0.9% saline and 4 ml of blood from study subjects to prevent binding of the drug to the infusion material. Exenatide was administered i.v. with a calibrated syringe pump at an infusion rate of 50 ng/min for 30 min, followed by 25 ng/min for the remainder of the test procedures, targeting steady-state plasma concentrations within the therapeutic range (100–125 pg/ml), as confirmed previously by Fehse et al.¹⁸ L-NMMA acetate (Bachem GmbH, Weil am Rhein, Germany) was diluted in isotonic 0.9% saline and primed over 5 min with a loading dose of 5 mg/kg, followed by a continuous infusion rate of 50 µg/kg/min until the end of the testing day, as this infusion protocol was shown to have vasopressor activity in healthy subjects.¹⁹

Biochemical analyses

Blood was drawn from the i.v. catheter using syringes, immediately transferred to designated BD Vacutainer® tubes (Franklin Lakes, NJ, USA) and centrifuged at 1370 g for 10 min at 4 °C. Plasma was separated and stored at –80 °C before assay. Heparin plasma and urine inulin and PAH were measured by colorimetric assay after preparation with p-dimethylamino-benzaldehyde (inulin)²⁰ or trichloroacetic acid and indole-3-acetic acid (PAH).²¹

Triglycerides and total cholesterol were determined using enzymatic colorimetric methods and HDL cholesterol was assessed using the third-generation HDL cholesterol plus method. LDL cholesterol was calculated using the Friedewald formula.²² Plasma and urinary sodium and potassium were measured by an indirect ion-selective electrode method, whereas urea was assessed by enzymatic colorimetric tests on a Modular-P auto-analyser. Urinary osmolality was determined by freezing point depression with a micro-osmometer (Fiske, Norwood, MA, USA). Urinary pH was measured using a hand-held VARIO® 2V00 pH-meter and SenTix-V electrode (Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany). Throughout the testing day, venous plasma glucose was measured using a YSI-2300 STAT Glucose analyser (YSI Life Sciences, Yellow Springs, OH, USA), while morning fasting glucose was measured from heparin plasma using the Gluco-Quant-hexokinase method on a Modular-P (Roche Diagnostics, Basel, Switzerland) <1 h after collection. Insulin was determined from heparin plasma using an immunometric assay (ADVIA Centaur-XP Immunoassay System; Siemens Healthcare, Erlangen, Germany). Updated homeostatic model assessment of insulin resistance (HOMA2-IR) was used to estimate insulin resistance from fasting glucose and insulin, and was obtained by the HOMA Calculator (<https://www.dtu.ox.ac.uk/homacalculator>).

Sample size and power calculation

The primary endpoint of this study was change in GFR after i.v. exenatide administration compared with isotonic 0.9% saline (placebo). Based on previous clinical data,⁶ we assumed that a paired t-test with a two-sided significance level of 0.05, a standard deviation (SD) of 8 mL/min, and a sample size of eight healthy overweight subjects, would provide 89% power to detect a mean difference in GFR of 9 mL/min. To allow for drop-outs and failed experiments, we included 10 healthy men in the study.

Statistical analyses

Data are presented as mean \pm SD or, in case of skewed distribution (based on visual inspection and the Shapiro–Wilk test), median [interquartile range (IQR)]. Log transformation of non-Gaussian distributed data to achieve normality before analysis was carried out. Statistical analyses were performed using appropriate parametric (paired t-test) and non-parametric tests (Wilcoxon signed-rank test), to identify single effects of exenatide versus placebo, and L-NMMA + exenatide versus exenatide. Pearson's correlation was used to determine associations between changes in renal haemodynamics and predefined variables that could theoretically influence renal vascular tone. For one subject, renal haemodynamic data during placebo and exenatide infusion periods were discarded because of failure of continuous i.v. administration of renal tracer substances; therefore, for these intervention periods, the data for nine subjects were used for renal haemodynamic analyses. Two-tailed p values of <0.05 were taken to indicate statistical significance. Analyses were performed using SPSS 20.0 (IBM SPSS Inc., Chicago, IL, USA).

Results

Renal haemodynamic effects

Exenatide infusion increased GFR by a mean \pm SD of 18 ± 20 mL/min/1.73 m² (+20%; $p=0.021$), whereas a numerically smaller but statistically significant rise in GFR was observed during combined exenatide and L-NMMA infusion compared with L-NMMA alone [mean \pm SD 8 ± 12 mL/min/1.73 m² (+9%; $p=0.049$); Figure 2]. Compared with placebo, exenatide

Table 1. Clinical and anthropometric characteristics

Characteristic	Healthy subjects (N = 10)
Age (years)	22.0 [22.0; 23.3]
BMI (kg/m ²)	29.4 \pm 1.7
Waist circumference (cm)	101.5 \pm 5.9
Fat percentage (%)	31.2 [26.6; 32.4]
Systolic BP (mmHg)	113 [111; 128]
Diastolic BP (mmHg)	65 [63; 69]
Fasting plasma glucose (mmol/L)	4.7 [4.3; 5.2]
2-h post-OGTT glucose (mmol/L)	5.7 [4.0; 6.2]
HbA _{1c} (mmol/mol)	32.0 [31.0; 33.3]
HOMA2-IR	0.69 [0.60; 1.75]
Triglycerides (mmol/L)	1.3 \pm 0.7
Total cholesterol (mmol/L)	4.3 \pm 0.9
HDL-cholesterol (mmol/L)	1.2 \pm 0.2
LDL-cholesterol (mmol/L)	2.5 \pm 0.8
Serum albumin (mg/L)	40.5 [39.0; 41.25]

Data are mean \pm standard deviation or median [interquartile range] values. BMI, body mass index; BP, blood pressure; HbA_{1c}, glycated haemoglobin; HOMA2-IR, updated homeostatic model assessment of insulin resistance; OGTT, oral glucose tolerance test.

increased ERPF by a median (IQR) of 68 (26; 197) mL/min/1.73 m² (+14%; p=0.015) and RBF by a mean ± SD of 183 ± 197 mL/min/1.73 m² (+23%; p=0.018), and reduced renal vascular resistance by 0.015 ± 0.015 mmHg/l/min (-14%; p=0.012). These effects were absent during simultaneous L-NMMA administration (Table 2), although a trend was observed for ERPF (p=0.093). Exenatide infusion did not alter filtration fraction on either of the two testing days. No significant differences in any of the variables were observed between the two urine collection periods per intervention condition (data not shown). A significant increase in estimated P_{GLO} [+3.8 (IQR 0.5; 7.3) mmHg; +6%; p=0.015] was noted after exenatide infusion, which was paralleled by a decrease in R_A (-33%; p=0.038) and no change in R_E (Table 2; Figure 3). Although both R_A and R_E were increased by L-NMMA (data not shown; expected based on previous data²³), subsequent exenatide administration did not yield any significant change in R_A or R_E.

Systemic haemodynamic effects

Systolic and diastolic BP and MAP did not change during exenatide versus placebo infusion, whereas exenatide increased systolic BP by a median (IQR) of 9.9 (2.8; 12.4) mmHg during concomitant L-NMMA infusion (p=0.007). Heart rate increased significantly after exenatide infusion on both testing days.

Metabolic effects

Plasma glucose consistently decreased after exenatide infusion (Table 2), although none of the healthy subjects developed symptoms of hypoglycaemia. Insulin concentrations were not affected by exenatide, in keeping with the glucose-dependent insulinotropic effect of the antihyperglycaemic drug. Body water remained constant throughout both testing days.

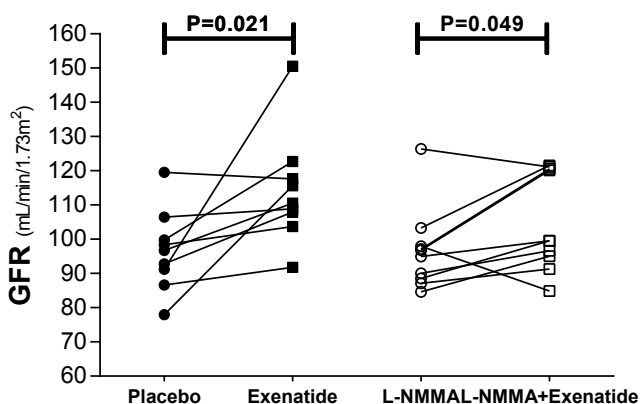


Figure 2. Glomerular filtration rate (GFR) responses to intravenous glucagon-like peptide-1 receptor agonists exenatide, with or without concomitant L-N^G-monomethyl arginine (L-NMMA) infusion.

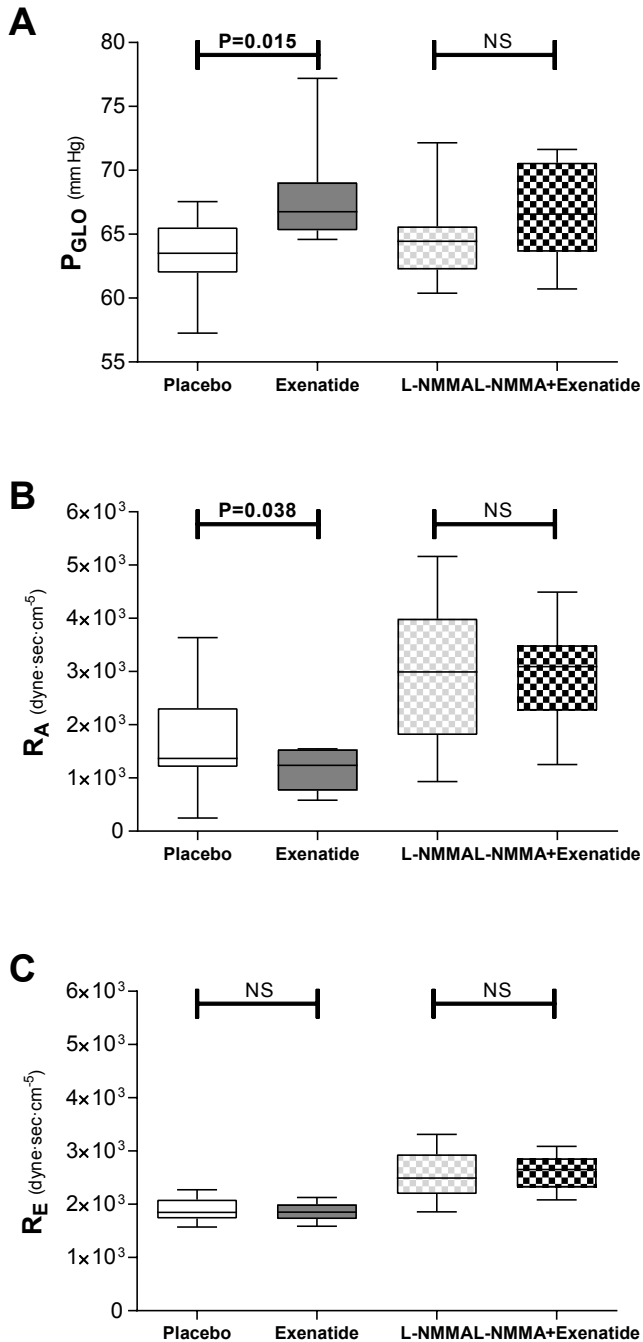


Figure 3. Estimated glomerular hydrostatic pressure (P_{GLO}) (A) and vascular resistance of afferent (R_A) (B) and efferent (R_E) (C) renal arterioles in response to intravenous glucagon-like-1 peptide receptor agonists exenatide, with or without concomitant L-N^G-monomethyl arginine (L-NMMA) infusion.

Table 2. Haemodynamic, tubular and metabolic responses to GLP-1RA exenatide-infusion, with or without concomitant L-NMMA-infusion

	Placebo	Exenatide	p*	L-NMMA	L-NMMA + Exenatide	p [#]
Renal haemodynamics						
GFR (mL/min/1.73 m ²)	97 ±12	114 ±16	0.021	97 ±12	105 ±14	0.049
ERPF (mL/min/1.73 m ²)	501 [434; 553]	585 [543; 607]	0.015	401 [313; 456]	408 [362; 465]	0.093
RBF (mL/min/1.73 m ²)	887 ±149	1070 ±179	0.018	692 ±118	727 ±109	0.179
RVR (mmHg/L/min)	0.095 ±0.017	0.080 ±0.010	0.012	0.137 ±0.036	0.139 ±0.043	0.885
FF	0.198 ±0.022	0.191 ±0.017	0.195	0.251 ±0.039	0.256 ±0.032	0.464
P _{cr,0} (mmHg)	63.5 [62.0; 56.5]	66.8 [65.3; 69.0]	0.015	64.5 [62.2; 65.6]	66.6 [63.6; 70.6]	0.074
R _A (dyne-sec-cm ⁻⁵)	1368 [1208; 2314]	1235 [760; 1536]	0.038	2990 [1809; 3993]	3092 [2266; 3493]	0.878
R _E (dyne-sec-cm ⁻⁵)	1845 [1734; 2087]	1854 [1720; 1998]	0.374	2495 [2192; 2938]	2652 [2319; 2862]	0.386
Haematocrit (L/L)	0.44 ±0.02	0.44 ±0.02	0.244	0.43 ±0.03	0.43 ±0.02	0.213
Renal tubular function						
Na excretion (μmol/min/1.73 m ²)	171.69 ±72.28	378.51 ±160.26	0.001	102.51 ±50.10	240.66 ±95.32	0.002
FF _{Na} (%)	1.34 ±0.46	2.45 ±0.91	0.001	0.75 ±0.34	1.60 ±0.73	0.005
FF _K (%)	17 ±4	17 ±7	0.984	17 ±7	16 ±5	0.090
FF _{urea} (%)	70 ±4	69 ±24	0.893	66 ±5	62 ±8	0.254
Urine osmolality	163 ±35	443 ±98	<0.001	196 ±66	408 ±95	<0.001
Urinary pH	6.53 [6.03; 6.90]	7.04 [6.89; 7.34]	0.005	6.32 [5.77; 6.72]	7.06 [6.50; 7.32]	0.005
Systemic haemodynamics						
Systolic BP (mmHg)	117 [107; 123]	119 [115; 124]	0.169	120 [113; 129]	127 [121; 132]	0.007
Diastolic BP (mmHg)	68 [62; 72]	65 [60; 70]	0.241	76 [69; 79]	75 [71; 80]	0.721
MAP (mmHg)	87 [77; 88]	84 [80; 87]	0.953	91 [86; 93]	92 [90; 96]	0.059
Heart rate (beats/min)	58 ±8	64 ±8	0.017	53 ±7	60 ±8	<0.001
Metabolic variables						
Plasma glucose (mmol/L)	4.31 [4.20; 4.43]	3.77 [3.63; 3.91]	0.005	4.40 [4.27; 4.68]	3.78 [3.70; 4.03]	0.005
Plasma insulin (mmol/L)	35.2 ±16.6	32.4 ±12.4	0.500	33.5 ±17.1	32.4 ±9.2	0.616
Body water (%)	51.5 ±2.2	51.2 ±2.2	1.000	51.4 ±2.0	51.1 ±1.6	0.299

Normally distributed variables are presented as mean ± standard deviation, and analysed using a paired t-test. Non-Gaussian distributed data are presented as median (interquartile range), and analysed using a Wilcoxon signed rank test. p values are based on statistical analyses performed on the comparison between (*) exenatide versus placebo; and (#) L-NMMA + exenatide versus L-NMMA. Significant values (p < 0.05) are shown in bold. BP, blood pressure; ERPF, effective renal plasma flow; FE, fractional excretion; FF, filtration fraction; GFR, glomerular filtration rate; L-NMMA, L-N^G-monomethyl arginine; MAP, mean arterial pressure; P_{cr,0}, glomerular hydrostatic pressure; RBF, renal blood flow; R_A, afferent renal arteriolar resistance; R_E, efferent renal arteriolar resistance; RVR, renal vascular resistance.

Tubular function effects

Absolute sodium excretion increased by a mean of $206.82 \mu\text{mol}/\text{min}/1.73 \text{ m}^2$ ($p=0.001$) from placebo after exenatide infusion. Fractional sodium excretion (FE_{Na}) increased from $1.34 \pm 0.46\%$ during placebo to $2.45 \pm 0.91\%$ during exenatide (+86%; $p=0.001$). Concomitant L-NMMA infusion did not affect exenatide-induced sodium excretion. Urinary osmolality and pH consistently increased after exenatide administration. No exenatide-induced effects on fractional potassium and urea excretion were observed.

Univariable correlations

Exenatide-induced changes in GFR and ERPF were not associated with alterations in MAP, heart rate, absolute sodium excretion, FE_{Na} , glucose or insulin concentrations ($p>0.05$). A non-significant negative correlation was observed between exenatide-induced changes in GFR and R_A ($R -0.586$; $p=0.098$). Changes in sodium excretion were not associated with MAP.

Discussion

The present mechanistic study is the first to provide insight into the acute effects of GLP-1 receptor agonist therapy on renal haemodynamics and tubular function in humans. We show that i.v. GLP-1 receptor agonist exenatide administration increases 'gold standard' measured GFR, ERPF and estimated P_{GLO} in healthy overweight men. These findings are probably explained by an exenatide-induced reduction in estimated R_A , which is at least partially dependent on NO availability. During L-NMMA, exenatide led to an isolated persistent increase in GFR, suggesting that this was caused by extrarenal effects, such as increased BP. In addition, we show that acute exenatide administration induces NO- and BP-independent natriuresis.

The complex mechanisms involved in the control of human renal haemodynamics comprise a variety of mechanisms that influence preglomerular (afferent) and postglomerular (efferent) resistance vessel tone. In our experiments, exenatide reduces estimated R_A , which could explain the observed increases in GFR, RBF and estimated P_{GLO} . These results are consistent with previous observations in preclinical studies, in which both systemic and intrarenal GLP-1 peptide⁷⁻⁹ and GLP-1 receptor agonist¹⁰ infusion increased GFR and RBF, probably as a result of relative vasodilation of preglomerular arterioles and consequently reduced renal vascular resistance.¹⁰

The present findings are in contrast with reported renal haemodynamic responses after GLP-1 peptide infusion in humans. GLP-1 infusion in 15 healthy lean subjects did not affect GFR estimated by creatinine clearance.⁶ Furthermore, in a study by Skov et al.,¹¹ GLP-1 infusion did not result in significant increases in radioisotope-measured GFR (+1.9%) and ERPF (+2.4%) in 12 healthy young males. Finally, synthetic human GLP-1 infusion had no effect on measured GFR and estimated ERPF in seven healthy men.¹² Notably, the latter two cited studies reached venous plasma GLP-1 concentrations of 87 mmol/L¹¹ and 65 mmol/L,¹² respectively. As we aimed for stable pharmacological GLP-1 receptor agonist concentrations with our validated infusion protocol,¹⁸ the exenatide-induced renal haemodynamic alterations

observed in the present study could be attributable to higher plasma concentrations, reflecting a clinically more relevant condition. Alternatively, as exenatide shares only 53% amino acid sequence homology with GLP-1 and is not degraded to the vasoactive GLP-1₍₉₋₃₆₎ metabolite,²⁴ clinical outcome dissimilarities between the two molecules were previously reported,^{13,14} and may account for the observed differential effect on renal haemodynamics in healthy humans.

Interestingly, in one study in healthy obese insulin-resistant volunteers, GLP-1 infusion reduced GFR from 151 to 142 mL/min, as measured by creatinine clearance.⁶ Notably, these subjects were characterized by significant glomerular hyperfiltration at baseline. Also, in a recent uncontrolled study in patients with T2DM, 7-week treatment with the GLP-1 receptor agonist liraglutide reduced ⁵¹Cr-EDTA-measured GFR by 11 mL/min/1.73 m².²⁵ After washout of the study-drug, GFR returned towards baseline values, suggesting reversible haemodynamic effects rather than structural changes; however, as no appropriate control group was included in this trial, the renal effects of GLP-1 receptor agonists in patients with T2DM remain uncertain.

The renal haemodynamic effect of exenatide may be direct and/or indirect in nature. A direct effect may be conceivable, as GLP-1 receptors are present in human and monkey smooth muscle cells of the afferent renal arteriolar wall.²⁶ In rodents, GLP-1-induced increases in RBF and reduced autoregulatory response in afferent renal arterioles were attenuated after GLP-1 receptor blockade with Exendin₍₉₋₃₉₎, suggesting that a direct GLP-1 receptor-mediated mechanism is involved;¹⁰ therefore, we postulate that, in the present study, direct GLP-1 receptor activation on renovascular smooth muscle cells could have led to exenatide-induced vasorelaxation, thereby reducing preglomerular vascular resistance and increasing GFR, ERPF and P_{GLO} in healthy overweight subjects.

Renal vascular actions of GLP-1(receptor agonists) may also be through indirect activation of local or systemic neurohormonal/vasoactive substances. First, as the vasodilating effect of exenatide on afferent renal arterioles was abolished during co-infusion with L-NMMA, we conclude that exenatide-induced effects are, at least in part, dependent on NO availability. Indeed, in rat and human arteries, GLP-1(receptor agonist) administration was found to induce endothelium-dependent vasodilation by increasing endothelial NO synthase phosphorylation and NO production via a 5'AMP-activated protein kinase-dependent pathway.²⁷ In addition, NO blockade abolished the vasodilator effect of GLP-1 in mesenteric mouse arteries;²⁴ however, the vasorelaxing effect of GLP-1(receptor agonists) also occurs through NO-independent mechanisms in a variety of vascular beds in rodent^{28,29} and human¹⁵ experiments. A study in normotensive rats showed that GLP-1-induced alterations in renal haemodynamics were not influenced by pharmacological blockade of NO.¹⁰ Since we observed an exenatide-induced increase in GFR during NO blockade, an NO-independent pathway cannot be rejected. These effects could, however, also be the result of exenatide-induced non-renal effects during L-NMMA, such as increased BP. Second, in the present study, exenatide reduced plasma glucose concentrations and did not affect insulin levels. Although both are known to influence renal vascular tone,³⁰ we did not observe a significant association with any renal haemodynamic change; however, to exclude the effects of exenatide-induced changes on glucose and insulin levels, in further studies these measurements could be performed during steady-state glucose

and insulin levels using clamp experiments. Third, the effects of GLP-1 (receptor agonists) on the renin-angiotensin-aldosterone-system, atrial natriuretic peptide, glucagon and sympathetic nervous system activity could have influenced the effects on renal haemodynamics. Indeed, these factors were implicated as mediators in acute and long-term renal effects of GLP-1 (receptor agonist) administration in humans,² but because the present study was not designed to assess the possible influence of these factors, further studies should investigate their role in the gut–renal axis. Finally, renal haemodynamics also adapt to changes in the salt concentration of distal tubular fluid through a mechanism known as tubuloglomerular feedback (TGF). An increase or decrease in sodium chloride concentration at the distally located macula densa elicits inverse changes in single-nephron GFR by altering the vascular tone of the afferent arteriole. In the present study, exenatide infusion increased urinary sodium excretion and decreased hydrogen excretion. These and previously reported findings^{2,6-9,11,25,31,32} support the concept that GLP-1 (receptor agonist) administration reduces Na⁺/H⁺-exchanger isoform-3 (NHE3) activity in the apical membrane of proximal tubular cells, thereby functioning as a proximal diuretic and urinary alkaliser. Theoretically, decreased proximal sodium reabsorption would initiate vasoconstriction of the preglomerular arteriole through TGF; however, the net effect of exenatide on preglomerular arterioles was vasodilation in the present study, suggesting a stronger direct vasodilative effect.

We hypothesise that the renal effect of exenatide in healthy men may differ from that in insulin-resistant subjects and patients with T2DM, which could explain the above-mentioned decrease in GFR after GLP-1 peptide infusion in these individuals. First, as NO-dependent vasodilation is often impaired in patients with T2DM,³³ the renal vasodilative effect of exenatide may theoretically be blunted. Furthermore, in these patients, GLP-1 receptor agonists may induce a more pronounced afferent vasoconstrictor effect through TGF. As increased proximal sodium reabsorption [as a result of increased NHE3 and sodium-glucose co-transporter (SGLT) protein expression and activity] in people with insulin resistance and T2DM leads to a baseline state of afferent arteriolar relaxation and glomerular hyperfiltration, this may allow a larger vasoconstrictive TGF response after induction of proximal natriuresis.² For example, an SGLT-2 inhibitor (which also functions as a proximal diuretic) reduced GFR³⁴ and increased R_A ³⁵ in hyperfiltering patients with type 1 diabetes (T1DM), probably by affecting TGF mechanisms, whereas no SGLT-2 inhibitor-induced renal haemodynamic effect was observed in normofiltering patients with T1DM; therefore, it could be suggested that GLP-1 (receptor agonist) administration only reduces GFR in the setting of hyperfiltration and/or diabetes, while it has no or increasing effect on GFR during normal baseline renal haemodynamic function and intact endothelial function.³⁶

The present study has some limitations. First, the sample size was relatively small, potentially leading to heterogeneity in renal haemodynamics at baseline and in individual responses to the interventions. We attempted to minimize the effect of a small sample size by conducting a careful preparation phase with emphasis on factors that influence neurohormonal activation, such as dietary sodium intake, and using a study design that allowed each subject to act as its own control. Second, our study population is not the target population for GLP-1 receptor

agonist treatment, indicating the need for renal studies in patients with T2DM in whom renal responses may theoretically be different; however, as liraglutide has now been endorsed for the treatment of obesity in individuals without diabetes, our findings of potentially deleterious effects on renal glomeruli could have important long-term clinical relevance and merits further study. Third, as the intervention conditions were performed in a sequential fashion, we were unable to exclude time-dependent effects. Finally, our estimation of glomerular characteristics with the Gomez formulae, by necessity, requires assumptions.

In conclusion, we showed that the GLP-1 receptor agonist exenatide increases gold standard-measured GFR, ERPF and estimated P_{GLO} in healthy overweight males, probably by reducing R_A , and at least partially in an NO-dependent manner. The clinical impact of our findings on renal outcome needs to be determined in long-term intervention trials in an overweight population. Furthermore, future studies should assess the acute and long-term renal effects of GLP-1 receptor agonists in patients with T2DM, as altered baseline renal haemodynamic function and subsequent renovascular responses may theoretically result in different responses.

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Supplemental methods

Calculation of intrarenal haemodynamic functions

Filtration pressure across the glomerular capillaries (ΔP_F) is calculated by the following Gomez-formula,¹ with the gross filtration coefficient (K_{FG}) assumed to be 0.0645 mL/sec/mmHg given a normal kidney physiology where GFR is 97 mL/min (mean GFR in the current population), glomerular hydrostatic pressure (P_{GLO}) is 60 mmHg (given Winton's indirect estimates in the dog that glomerular pressure is roughly two-thirds of MAP²), and normal glomerular oncotic pressure (π_G) is 25 mmHg:

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

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

$$C_M = TP/FF * \text{Ln} (1/1 - FF)$$

$$\pi_G = 5 * (C_M - 2)$$

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

$$P_{GLO} = \Delta P_F + P_{BOW} + \pi_G$$

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

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

$$R_A = [(MAP - P_{GLO} / RBF) * 1328]$$

$$R_E = [GFR / (K_{FG} * (RBF - GFR))] * 1328$$

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