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ORIGINAL RESEARCH

GLP–1 Promotes Cortical and Medullary Perfusion in the Human Kidney and Maintains Renal Oxygenation During NaCl Loading

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BACKGROUND: GLP-1 (glucagon-like peptide-1) receptor agonists exert beneficial long-term effects on cardiovascular and renal outcomes. In humans, the natriuretic effect of GLP-1 depends on GLP-1 receptor interaction, is accompanied by suppression of angiotensin II, and is independent of changes in renal plasma flow. In rodents, angiotensin II constricts vasa recta and lowers medullary perfusion. The current randomized, controlled, crossover study was designed to test the hypothesis that GLP-1 increases renal medullary perfusion in healthy humans.

METHODS AND RESULTS: Healthy male participants (n=10, aged 27±4 years) ingested a fixed sodium intake for 4 days and were examined twice during a 1-hour infusion of either GLP-1 (1.5 pmol/kg per minute) or placebo together with infusion of 0.9% NaCl (750 mL/h). Interleaved measurements of renal arterial blood flow, oxygenation (R_2*), and perfusion were acquired in the renal cortex and medulla during infusions, using magnetic resonance imaging. GLP-1 infusion increased medullary perfusion (32±7%, P<0.001) and cortical perfusion (13±4%, P<0.001) compared with placebo. Here, NaCl infusion decreased medullary perfusion (–5±2%, P=0.007), whereas cortical perfusion remained unchanged. R_2* values increased by 3±2% (P=0.025) in the medulla and 4±1% (P=0.008) in the cortex during placebo, indicative of decreased oxygenation, but remained unchanged during GLP-1. Blood flow in the renal artery was not altered significantly by either intervention.

CONCLUSIONS: GLP-1 increases predominantly medullary but also cortical perfusion in the healthy human kidney and maintains renal oxygenation during NaCl loading. In perspective, suppression of angiotensin II by GLP-1 may account for the increase in regional perfusion.

REGISTRATION: URL: https://www.clinicaltrials.gov; Unique identifier: NCT04337268.

Key Words: arterial spin labelling ■ BOLD ■ GLP-1 ■ kidney ■ magnetic resonance imaging ■ perfusion ■ RBF ■ renal

Recent cardiovascular outcome trials demonstrated beneficial cardiovascular actions of glucagon-like peptide-1 (GLP-1) receptor agonists used in patients with type 2 diabetes at high cardiovascular risk.[1,2] A comprehensive review suggested that among patients with type 2 diabetes, GLP-1 receptor agonists, and to a lesser extent dipeptidyl peptidase-4 inhibitors, in addition to standard treatment of diabetes modestly improve albuminuria, plausibly beyond the effects of glycemic control and halted estimated glomerular filtration rate

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CLINICAL PERSPECTIVE

What Is New?

- GLP-1 (glucagon-like peptide-1) increases mainly renal medullary perfusion but also cortical perfusion and renal oxygenation during NaCl loading.

What Are the Clinical Implications?

- By preserving renal tissue oxygenation, improved renal perfusion may contribute to the long-term beneficial renal and cardiovascular effects of GLP-1 receptor agonists.

Nonstandard Abbreviations and Acronyms

ANGII	angiotensin II
GLP-1	glucagon-like peptide-1

decline in patients with a high risk of cardiovascular and renal events.[3] Thus, the beneficial cardiovascular effect of GLP-1 appears to be partly related to renoprotection.

In human studies, native GLP-1, as well as GLP-1 receptor agonists, elicit acute natriuresis in healthy young male participants, insulin-resistant obese participants, and in patients with type 2 diabetes.[4–10] Extracellular fluid volume expansion (with isotonic saline infusion) uncovers this natriuretic action of GLP-1, probably mediated by a tubular mechanism in distal nephron segments.[5,6,11] GLP-1–mediated natriuresis was associated with suppression of plasma angiotensin II (ANGII) concentration, but not renin, and is coupled to a high renal extraction of GLP-1.[5,6,11] In contrast to rodents,[9] this natriuretic action in humans is independent of changes in total renal plasma flow and glomerular filtration rate and is not related to GLP-1–induced changes in plasma atrial natriuretic peptide concentration.[5] The mechanism by which GLP-1 reduces ANGII concentration has not been clarified, but it depends on GLP-1 receptor activation.[6] ANGII potently constricts efferent arterioles and vasa recta.[12–15] Endogenous ANGII has been shown to tonically decrease medullary blood flow in rodents.

The present study was designed to investigate the hypothesis that GLP-1 increases renal medullary perfusion and oxygenation. Because transepithelial Na^+ transport is the dominant process for oxygen consumption in the kidneys, the hypothesis was tested during acute Na^+ loading. Ten healthy male participants were recruited and ingested a standardized sodium intake to equalize renin-angiotensin-aldosterone system activity and received GLP-1 or placebo infusions during magnetic resonance imaging (MRI) recordings of renal arterial blood flow, perfusion, and oxygenation (R_2^*) in a randomized, controlled, crossover design study. The MRI technique was applied because it allows determination of dynamic changes in these parameters in both kidney medulla and cortex and to give insight into differential regional changes in perfusion and tissue oxygenation.

METHODS

Data Availability

The authors declare that all supporting data are available within the article.

Participants

Baseline characteristics are shown in Table 1. Ten young male participants of Caucasian origin completed all components of the study. All participants were healthy, and none took medication at the time of the study. Body composition was determined by dual-energy x-ray absorptiometry scanning (Lunar iDXA; GE Healthcare, Brøndby, Denmark) (Table 1). Consent to participate was obtained after the participants had read a description of the experimental protocol in accordance with the Declaration of Helsinki. The protocol was approved by the Scientific Ethics Committee of the Capital Region of Copenhagen (H-18050603) and is registered as a clinical trial (ClinicalTrials.gov identifier: NCT04337268).

Table 1. Baseline Characteristics

Variable	Value
Age, y	27±4
Height, cm	183±7
Weight, kg	76.6±7.2
Lean body mass, kg	62.4±6.9
Whole body fat mass, kg	14.6±3.6
Systolic blood pressure, mm Hg	116±11
Diastolic blood pressure, mm Hg	73±7
Heart rate, bpm	63±7
Glucose, mmol/L	5.4±0.4
HbA1c, mmol/mol	34.1±2.5
Hemoglobin, mmol/L	9.3±0.5
Creatinine, µmol/L	80±8
eGFR, mL/min	>90
Alanine transaminase, U/L	26±11
Aspartate aminotransferase, U/L	27±6

Body composition was determined by dual-energy x-ray absorptiometry scanning. Blood samples were taken under fasting conditions, and eGFR was calculated, using the Chronic Kidney Disease Epidemiology Collaboration equation. Data are presented as mean±SD. eGFR indicates estimated glomerular filtration rate; and HbA1c, hemoglobin A1c.

Experimental Design

The study protocol had a single-blinded, randomized, controlled, crossover design with a washout period of ≈4 weeks (Figure 1A). Each participant served as his own control and was studied on 2 different occasions during a 1-hour infusion of GLP-1 (1.5 pmol/kg per minute) with coinfusion of 0.9% NaCl (750 mL/h) or a 1-hour infusion of placebo with coinfusion of 0.9% NaCl (750 mL/h) (Figure 1B). This resulted in arterial plasma concentrations of GLP-1 of ≈140 pmol/L during steady state, which is a ≈3-fold higher plasma level compared with steady state postprandial plasma levels and thus in the slightly supraphysiological range.[5,6,11,16,17]

Protocol

For 4 days before each experiment, all participants consumed a controlled mixed diet (2822 kcal per day; 16% protein, 55% carbohydrate, 29% fat). The food was handed out frozen, and the NaCl content of the diet, measured at Eurofins Stein's Laboratory in Denmark, was 55 to 75 mmol per day. NaCl was added to the diet to standardize daily intake at 2 mmol NaCl per kilogram of body weight per day. Twenty-four-hour urine was collected on the last day. Urinary sodium and potassium concentrations as well as pH were determined. Water intake was ad libitum, and strenuous physical activity was not allowed during the 4-day period.

Participants arrived in the morning hours having fasted for 12 hours before the beginning of the experiments. The experimental setup is shown in Figure 1B. After confirmation of an empty bladder by ultrasound, participants remained supine throughout the experiments and were given tap water (14 mL/kg, maximum 1000 mL), which was ingested within 10 minutes to keep participants in surplus of free water and thus suppress and maintain plasma vasopressin concentration at a constant low level. Meanwhile, a forearm vein was catheterized with an 18-gauge catheter (BD Venflon; 1.2 mm OD, length 45 mm; Becton Dickinson, Helsingborg, Sweden) for infusions as well as blood sampling. An intravenous infusion of 0.9% NaCl was administered (750 mL/h for ≈3 hours) to expand the extracellular fluid volume throughout the experiments, similar to our previous studies.[5,6]

Scanning Protocol

After ≈1.5 hours of 0.9% NaCl infusion, participants were transferred to the MRI scanner, maintaining the supine position. MRI measures began 30 minutes after transfer for a total of 2 hours infusion before scanning.

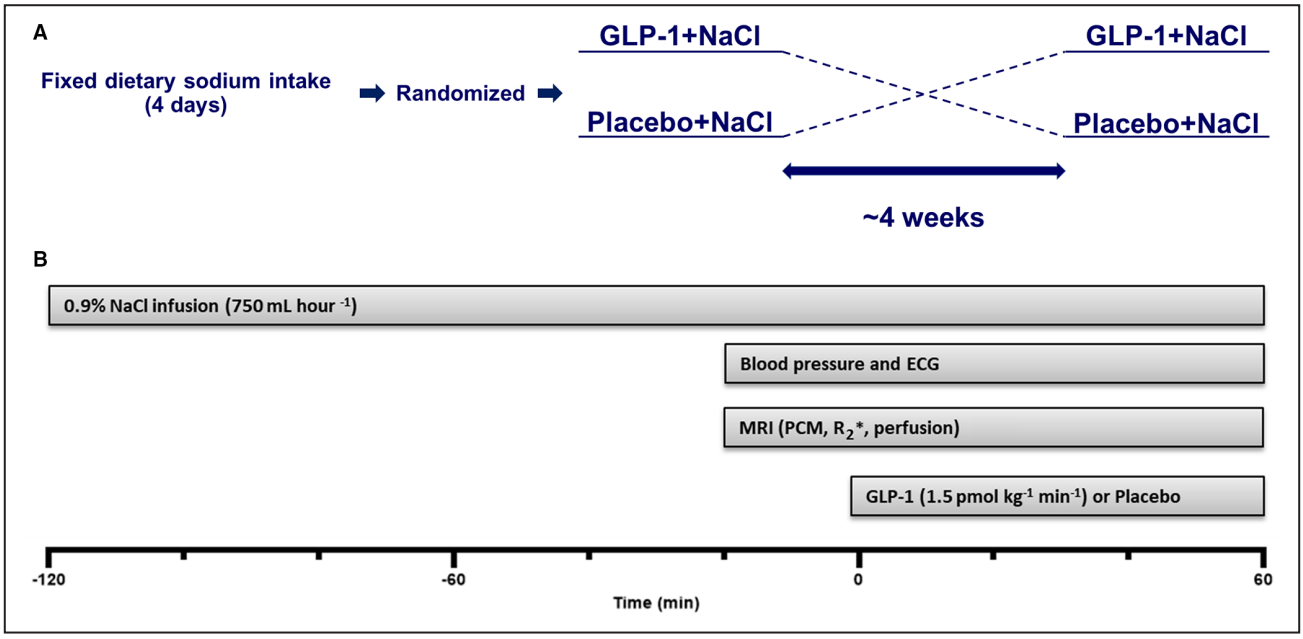

Figure 1. Study design and experimental timeline.
A, Study design. The study protocol had a single-blinded, randomized, controlled, crossover design with a washout period of around 4 weeks. Each participant served as his own control and was studied on 2 different occasions during a 1-hour infusion of GLP-1 (1.5 pmol/kg per minute) with coinfusion of 0.9% NaCl (750 mL/h) or a 1-hour infusion of placebo (saline) with coinfusion of 0.9% NaCl (750 mL/h). **B**, Experimental timeline. After emptying the bladder at time –120 minutes, participants remained supine throughout the experiments. Tap water (14 mL/kg, maximum 1000 mL) was ingested from time –120 to –110 minutes. Blood was sampled for blood glucose measurements at time 0 and 60 minutes. Interleaved measurements of renal arterial blood flow (PCM), oxygenation (R_2*), and perfusion were acquired in the renal cortex and medulla, using MRI from time –20 to 60 minutes. GLP-1 indicates glucagon-like peptide-1; MRI, magnetic resonance imaging; and R_2*, oxygenation.

Subsequently, a baseline blood sample was drawn, and blood pressure and heart rate were measured. Baseline measurements of renal perfusion, R_2*, and renal arterial blood flow data were acquired, followed by the start of a 1-hour infusion of either native GLP-1 (1.5 pmol/kg per minute) or placebo combined with the continued 750 mL/h saline infusion. The placebo used was 0.9% NaCl, having the same volume and being infused by the same pump as during the GLP-1 infusion. The solutions were prepared freshly. Participants were blinded with respect to the content. Renal perfusion, R_2*, and renal arterial blood flow were repeatedly acquired throughout the infusions along with blood pressure and heart rate measurements.

Scanning was performed on a 3T Philips Achieva scanner, using the scanner's body transmit coil and a 4-element SENSE anterior receive coil. Participants were scanned, using MRI sequences to map perfusion, R_2* in both kidneys, and to measure blood flow in the renal artery of the right kidney. All 3 measurements were acquired consecutively in a 7- to 10-minute block (dependent on the participants rate of respiration), which was performed at baseline before the intervention and repeatedly during the intervention, as shown in Figure 1B. Participants were permitted to pass urine if needed, where scanning was halted and the participant's urine was collected while remaining supine on the scanner table. Blood pressure, blood oxygenation, and heart rate were measured, using a Veris Monitor system (MEDRAD, Pittsburgh, PA). The total scan time was ≈75 minutes.

Intravenous GLP-1 Infusions

Synthetic human GLP-1 (7–36)amide was purchased from Bachem (Bubendorf, Switzerland) and demonstrated to be ≥97% pure and identical to the natural human peptide by high-performance liquid chromatography, mass, and sequence analysis. GLP-1 (7–36) amide was dissolved in saline containing 0.5% human serum albumin (CSL Behring, Marburg, Germany).

The infusion protocol used in the present study is identical to numerous previous human studies, in which slightly supraphysiological circulating GLP-1 levels reached steady state within 20 minutes during GLP-1 infusion. Under these conditions, a high renal GLP-1 extraction of ≈45% has been established.[5,6,11,16]

MRI: Perfusion, Arterial Spin Labeling

Acquisition parameters and postprocessing was performed in accordance with expert consensus-based recommendations for renal arterial spin labeling MRI and have been verified in previous studies.[18–21] Data were collected, using a respiratory-triggered FAIR labeling scheme with a postlabel delay of 1100 ms and a balanced fast field echo readout scheme (repetition time 3.2 ms/echo time 1.6 ms, flip angle 60°, SENSE factor 2, and linear acquisition).[22] The matrix size was 170×170, covering an field of view of 340 mm×340 mm with a 5-mm slice thickness. Acquisition parameters used for acquiring arterial spin labeling, T_1 maps, and M_0 data are described in Gardener et al.[23] A base equilibrium M_0 scan and T_1 map (inversion time values of 200–1300 ms [100-ms steps] and 1500 ms) were acquired, using respiratory triggering to allow arterial spin labeling perfusion quantification. All MRI data were motion corrected and were postprocessed, including the drawing of regions of interest (ROIs), independently by 2 observers who were blinded to the administration of GLP-1 or placebo. Data presented are the mean results from the 2 observers.

After motion correction, the mean perfusion weighted difference of the control-label pairs of images was quantified on a voxel-by-voxel basis, using the simplified perfusion model, neglecting transit time effects and exchange time[18,24]:

$$f = \frac{\lambda}{2\alpha TI}\frac{\Delta M(TI)}{M_0}\exp\left(\frac{TI}{T_1}\right)$$

where M_0 is tissue equilibrium magnetization, T_1 is the longitudinal relaxation in seconds, f is the perfusion rate in milliliters per 100g/min, and λ is the blood-tissue partition coefficient, assumed to be 0.9 mL/g for kidneys.[18] The labeling efficiency was assumed to be 0.9.

MRI: Oxygenation, R_2*

Single-slice, 2-dimensional, multiecho fast-field echo images were acquired, using the same field of view and matrix size as the arterial spin labeling data with a SENSE factor of 1.5, a repetition time of 48 ms, and a flip angle of 30°. A total of 8 echoes were acquired per measurement, with an initial echo time of 3 ms and echo spacing of 8-ms steps. All 8 echoes were acquired in a single breath hold of ≈15 seconds. R_2* maps were calculated, using a least squares minimization of the equation:

$$S_{(TE)} = S_0 \cdot e^{-TE \cdot R_2^*}$$

MRI: Renal Arterial Blood Flow

Blood flow in the right renal artery was calculated, using phase contrast MRI, following recommendations from expert consensus.[25] A single slice placed perpendicular to the renal artery of the right kidney was acquired, using respiratory and cardiac gating to image 12 time points of the cardiac cycle. Velocity encoding of 100 cm/s, a repetition time/echo time of 4.9/2.9 ms, and fast field echo acquisition were used with acquisition voxel dimensions of 2.5×2.5×8 mm^3 reconstructed to 2×2×8 mm^3. Renal artery blood flow was calculated from acquired images by drawing ROIs including the entire area of the renal

artery cross-section multiplied with its mean velocity for each frame of the cardiac cycle.

MRI: Data Analysis

Reported values of cortical and medullary perfusion and R_2* are mean values from ROIs drawn on both kidneys by a blinded observer. ROIs were drawn to include as much as possible of the entire volume of tissue excluding artifacts. Medullary ROIs were drawn to cover the entire medullary volume. All image manipulations, coregistrations, calculations, and statistical analyses were performed, using scripts created in MATLAB 2013b (MathWorks, Natick, MA).

Blood and Urine Analyses

Samples of blood were drawn immediately before the commencement of GLP-1 or placebo infusion and at the end of infusions. Both blood samples were analyzed for glucose, using an automated benchtop blood analyzer system (ABL 700 series; Radiometer Medical Aps, Brønshøj, Denmark).

Twenty-four-hour urinary sodium and potassium concentrations were measured by atomic absorption (atomic absorption spectrophotometer model 2380; PerkinElmer, Norwalk, CT), and urinary pH was measured, using a XC161 Combination pH electrode (Radiometer Medical Aps).

The GLP-1–dependent ANGII suppression and natriuretic effect have previously been clearly demonstrated under identical conditions as applied in the present study[5–7]; hence, we chose not to repeat these measurements to allow more continuous MRI scanning and thereby maintain a high time resolution on MRI parameters.

Statistical Analysis

The primary end point in the present study was renal medullary perfusion. When using a 2-tailed α=0.05 and requiring an 80% power threshold, the sample size of n=8 was calculated as required to detect an appreciable effect (>10% change) of exogenous GLP-1 on renal medullary perfusion. This calculation was based on previous human experiments with repeated measures of renal medullary perfusion having a coefficient of variation of 11%.[21]

A mixed model analysis was performed to test for changes in renal blood flow, cortical perfusion, cortical R_2*, medullary perfusion and medullary R_2*, and blood pressure and heart rate with intervention (GLP-1 versus placebo), and time after infusion start as fixed effects with the participant as a random effect (random intercept). Likewise, differences in baseline values (measures before intervention) were tested, using the day GLP-1 was administered versus the day placebo was administered as fixed effects.

A second mixed model analysis compared changes instigated by GLP-1 infusion from those in response to placebo, correcting for the variance within the individual participants as a random factor.

RESULTS

Standardized Sodium Chloride Intake

All participants completed the mixed controlled diet with fixed NaCl intake for 4 days before each experiment. On the last day of the 4-day period, all 24-hour urine collections were successfully completed with similar mean collection time on each study day (23.1±0.6 versus 24.0±0.8 hours, P=0.715). Twenty-four-hour urine data were statistically similar (Table 2).

Blood Glucose Concentration

At baseline, blood glucose levels were similar between the 2 study days (5.4±0.1 versus 5.3±0.1 mmol/L, P=0.5). After 1-hour GLP-1 infusion, blood glucose concentration decreased from baseline (4.1±0.09 versus 5.4±0.1 mmol/L, P<0.001) and remained unchanged 1 hour after placebo infusion (5.3±0.1 versus 5.2±0.1 mmol/L, P=0.4). None of the participants developed symptoms of hypoglycemia.

MRI Measurements of Functional Kidney Parameters at Baseline

Baseline measures were taken when participants had received 750 mL/h saline infusion in the supine position for 2 hours and were considered to be in a steady state. Baseline MRI measures of inner and outer medullary and cortical perfusion, cortical and medullary R_2*, and renal arterial blood flow (Table 3) did not differ significantly between the 2 study days. An average of 6.5 intervention measures were obtained for each MRI parameter during GLP-1 infusion compared with 5.8 time points during placebo. MRI measures for all participants were linearly interpolated to 6 time points of 10-minute intervals during the infusions. The variations at the 2-hour saline infusion baseline time point between the 2 scanning days for participants as a coefficient of variation are 14% and 10% for perfusion measure in the medulla and cortex, respectively, 7.7%

Table 2. Twenty-Four-Hour Urinary Excretions During Baseline

Twenty-four-hour urine variable	Baseline (GLP-1)	Baseline (saline)	*P* value
Volume, mL	2067±251	2107±289	0.922
Sodium, mmol	179±15	174±39	0.902
pH	6.6±0.2	6.5±0.2	0.604
Potassium, mmol	69±5	68±4	0.823

Data are presented as mean±SE. *P* values were obtained from mixed models. GLP-1 indicates glucagon-like peptide-1.

Table 3. Baseline Magnetic Resonance Imaging Measurements

Variable	Baseline (GLP-1)	Baseline (placebo)	*P* value
Cortical perfusion, mL/100 g per min	303±22	324±19	0.402
Outer medullary perfusion, mL/100 g per min	90±13	92±13	0.112
Inner medullary perfusion, mL/100 g per min	73±13	81±10	0.524
Cortical R_2*, s^{-1}	22±1	21±1	0.161
Medullary R_2*, s^{-1}	39±2	36±1	0.112
Renal arterial blood flow, mL/min	506±26	521±24	0.550

Baseline measures were timed such that participants were in a steady state of volume expansion, having received saline infusion (750 mL/h) in the supine position for 2 hours before scanning regardless of the intervention (GLP-1 or placebo infusion) that followed. Data are presented as mean±SEM. *P* values were obtained from mixed models. GLP-1 indicates glucagon-like peptide-1; and R_2*, oxygenation.

and 6.7% for the medullary and cortical R_2*, respectively, and 6.1% for renal arterial blood flow.

MRI Measurements of Functional Kidney Parameters: Response to GLP-1 and Placebo Intervention Compared With Preintervention Volume Expanded Baseline

During GLP-1 infusion, perfusion increased significantly (*P*<0.001) in the cortex, outer medulla, and inner medulla. During placebo infusion, perfusion of the cortex decreased significantly (*P*<0.007), whereas medullary perfusion did not change significantly (Table 4, Figure 2). There was no linear correlation with the duration of GLP-1 infusion and the induced perfusion change. During GLP-1, R_2* remained unchanged in the cortex and medulla, whereas during placebo, R_2* significantly increased, indicative of reduced blood oxygenation in the cortex (*P*=0.008) and medulla (*P*=0.025) (Table 4, Figure 3). An example of perfusion and R_2* parametric images for 1 participant is presented in Figure 4. Renal arterial blood flow did not change significantly after either GLP-1 infusion or placebo (Table 4, Figure 2). Mean relative changes in perfusion, R_2*, and renal arterial blood flow during infusions are presented in Table 4. When comparing the infusion of GLP-1 with placebo, perfusion in the inner medulla increased by 15±3% (*P*<0.001), perfusion in the outer medulla increased by 9±2% (*P*<0.001), and perfusion in cortex increased by 9±1% (*P*<0.001), relative to the placebo (Table 4, Figure 2). In contrast, the increase in cortical R_2* values during placebo exceeded cortical R_2* changes after GLP-1 infusion by 2±1% (*P*=0.022), with no significant differences in medullary R_2* changes between interventions (Table 4). Renal arterial blood flow values did not differ significantly during GLP-1 infusion compared with placebo (Table 4).

Effect of Intervention on Blood Pressure and Heart Rate

Systolic blood pressure did not change significantly at any intervention (data not shown). Diastolic blood pressure increased significantly by 2.9 mm Hg during placebo (4.5±1.0%, *P*=0.018) and 2.5 mm Hg during the same time period with GLP-1 infusion (3.4±1.7%, *P*=0.001). Heart rate increased 3.8 bpm during GLP-1 infusion (6.0±1.5%, *P*<0.001) and 2.6 bpm during placebo (4.2±1.6%, *P*=0.003). Changes in blood pressure and heart rate were not significantly different during GLP-1 infusion compared with placebo.

DISCUSSION

The present set of data support the hypothesis that renal cortical perfusion, and to a larger extent medullary

Table 4. Mean Changes in Magnetic Resonance Imaging Measurements During the 60-Minute Intervention

Variable	GLP-1	Saline (placebo)	*P* value GLP-1 vs placebo
Cortical perfusion, mL/100 g per min	13±4%*	−5±2%†	<0.001
Outer medullary perfusion, mL/100 g per min	30±4%*	7±5%	<0.001
Inner medullary perfusion, mL/100 g per min	32±7%*	−1±4%	<0.001
Cortical R_2*, s^{-1}	0±1%	4±1%†	0.022
Medullary R_2*, s^{-1}	1±1%	3±2%†	0.214
Renal arterial blood flow, mL/min	−1±2%	1±2%	0.537

Mean changes (± SEM) in perfusion, R_2*, and renal arterial blood flow during a GLP-1 and a placebo intervention. R_2* (indicator of deoxyhemoglobin) increases with declining tissue oxygenation. *P* values were obtained from mixed models. Preintervention values are baseline measurements where participants were already in a steady state of volume expansion, having received saline infusion (750 mL/h) in the supine position for 2 hours. The column GLP-1 vs placebo are the *P* values from the mixed-model analysis for differences between the response to GLP-1 and placebo interventions. GLP-1 indicates glucagon-like peptide-1; and R_2*, oxygenation.

*Significant difference between baseline and intervention values; *P*<0.001.

†*P*<0.05.

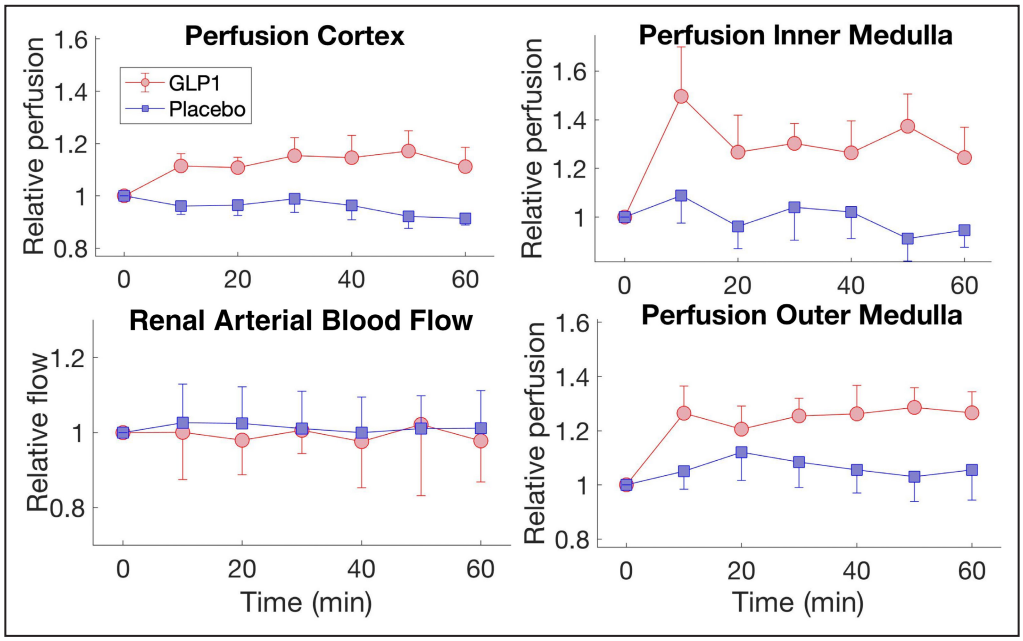

Figure 2. Relative changes in renal hemodynamics during GLP-1 and placebo intervention. Perfusion in the cortex, inner, and outer medulla increased significantly from baseline during the GLP-1 intervention and was significantly higher than during the placebo intervention. There were no significant variations of renal arterial blood flow from baseline during either intervention. Baseline measures (time=0) were timed such that participants had received 750 mL/h saline infusion for 2 hours in the supine position before the baseline scans and were in a steady state. After the baseline scans, the intervention of GLP-1 or placebo infusion commenced along with the continued 750 mL/h saline infusion. All measures were acquired repeatedly during the 60-minute intervention and interpolated to 10-minute intervals. Data are presented as mean±SEM. GLP-1 indicates glucagon-like peptide-1.

perfusion, increase significantly in response to physiologically relevant increase in plasma GLP-1 levels in volume-loaded healthy participants. Renal arterial blood flow was not affected by GLP-1 infusion despite the increases in regional tissue perfusion. The NaCl loading and extracellular fluid volume expansion increased R_2^* values during placebo, indicating reduced tissue oxygenation, whereas R_2^* values remained unchanged during GLP-1. Thus, GLP-1 attenuated the decrease in tissue oxygenation seen during NaCl infusion in accordance with the increase in perfusion. Likely, the sodium loading led to an increase in oxygen consumption because of increased metabolic tubular activity. Renal oxygen consumption is highly correlated to sodium transport rate. The observed increase in medullary perfusion agrees well with suppression of plasma ANGII concentration associated with the natriuretic effect of GLP-1, previously demonstrated under identical controlled conditions as applied in the present experiments.[5,6]

The present study supports a potentially beneficial alteration in regional renal perfusion in a state when the balance of oxygen demand versus delivery is challenged by volume loading. Harmful hypoxia can develop if oxygen demand increases or oxygen delivery is reduced, which is often the case in diabetes and hypertension or more severe in prerenal acute kidney injury.[26]

This study was performed during volume expansion with saline, which is a prerequisite to uncover the natriuretic effect of GLP-1 in humans.[5,11] Saline volume expansion induces natriuresis and reduces cortical perfusion by 10% to 20%[27,28], an action that we show is reversed by GLP-1. Renal vasoconstriction is unique to chloride-containing volume expansion.[29] Perfusion and oxygenation remain stable or even increase during volume expansion with solutions that are isochloraemic or free of chloride.[27,30–32] Chloride gradients and chloride channels are prerequisites for direct ANGII-induced vasoconstriction in the renal medullary vascular bed,[33,34] and chloride is the rate-limiting ion in the tubuloglomerular feed-back response.[35]

Renal medullary perfusion is a key contributing factor in control of renal sodium excretion.[36] In the present study, a contributory role of increased medullary perfusion in the well-established natriuretic effect of GLP-1 is possible via increased renal interstitial hydrostatic pressure at first with a subsequent crucial role in washout of solutes from the renal medullary interstitium and reduced sodium tubular reabsorption. In rats, renal medullary interstitial infusion of bradykinin increased medullary blood flow by 17% and doubled sodium

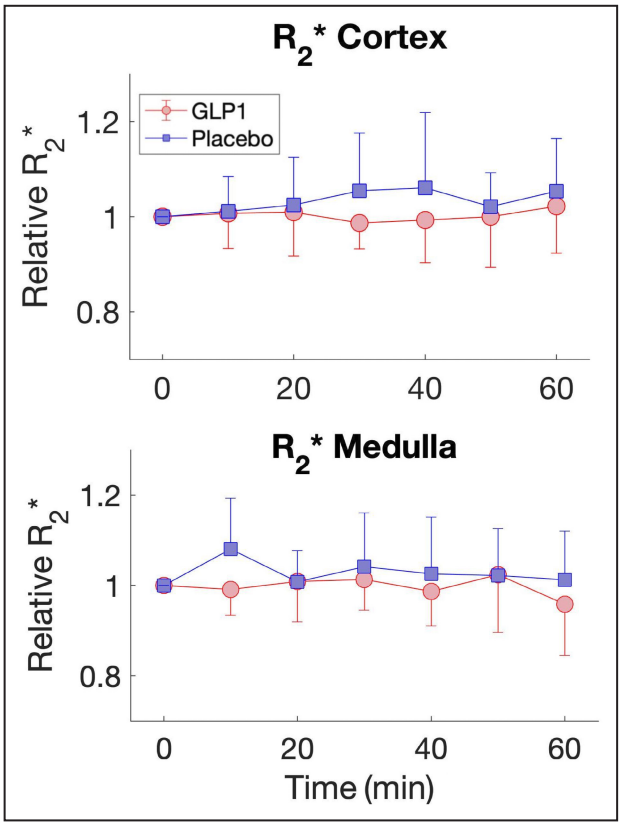

Figure 3. Relative changes in R_2* during GLP-1 and placebo intervention.
R_2* (indicator of deoxyhemoglobin) increases in the cortex and medulla were significant during placebo, whereas there were no significant changes during the GLP-1 intervention. Baseline measures (time=0) were timed such that participants had received 750 mL/h saline infusion for 2 hours in the supine position before the baseline scans and were in a steady state. After the baseline scans, the intervention of GLP-1 or placebo infusion commenced in addition to the continued 750 mL/h saline infusion. Data are presented as mean±SEM. GLP-1 indicates glucagon-like peptide-1; and R_2*, oxygenation.

excretion without altering renal blood flow or GFR.[37] In previous human studies, conducted under similar conditions as applied in the present study, GLP-1 suppressed plasma ANGII significantly and increased natriuresis.[5,6] In the present study, renal arterial blood flow remained unchanged on both study days in line with previous studies, demonstrating that GLP-1's natriuretic effect is independent of changes in net renal plasma flow and GFR as measured via Fick principle.[5,6]

There are few data in regard to the human kidney on GLP-1 receptor localization, although it has been demonstrated in rat smooth muscle cells of the preglomerular vasculature.[38] GLP-1 receptor activation leads to cAMP formation. If receptors were present in preglomerular vessels in humans, a preglomerular vasodilation would be predicted.[39,40] In rodents, GLP-1 leads to a dose-dependent preglomerular vasodilation. However, in animal experiments, GLP-1 levels are typically increased 100-fold above physiological levels compared with a 10-fold increase in human studies.[40] Activation of GLP-1 receptors is crucial for GLP-1-mediated suppression of plasma ANGII concentration.[6] In humans, pharmacological levels of ANGII reduce MRI-measured renal blood flow,[41] and previous data from rodents are consistent with a tonic vasoconstrictor effect of ANGII on medullary resistance vessels.[15] Therefore, GLP-1–associated ANGII suppression in humans may have increased renal medullary perfusion in the present study.

Mean arterial pressure increased slightly, driven by diastolic pressure in response to GLP-1. A simultaneous GLP-1–induced increase in cardiac output was proportionally greater because of significant vasodilation in skeletal muscle and adipose tissue.[17]

There are some limitations to be considered when interpreting the results of this study. In the present study, renal arterial blood flow remained unchanged despite changes in cortical and medullary perfusion during GLP-1 infusion. Although medullary perfusion only constitutes a small fraction of the total renal blood supply, a close relationship between changes of cortical perfusion and renal arterial blood flow would be expected. In humans, paradoxical changes (≈20%) in cortical perfusion concurrent with no significant decrease in renal arterial blood flow have been reported in MRI studies, measuring changes induced by applying extracellular fluid volume.[28,32] One possible explanation for this paradox could be coinciding changes in kidney volume, which was not monitored in this study. It is also important to point out that perfusion measures, using arterial spin labeling are not sensitive to changes in macrovascular flow. For this reason, although the entire contents of the renal artery pass through the cortex, measured changes in perfusion will predominantly reflect microvascular changes in cortical tissue, not total flow. A second physiological consideration is that baseline MRI parameters serve well as a preintervention measurements after participants have undergone ≈100 minutes of saline infusion and are not a true resting-state baseline. The primary strength of the crossover design is that effects of extracellular fluid volume in the study timeframe with and without GLP-1 can be compared, thus limiting confounding factors.[42] Although the changes in R_2* are presumed to be caused by changes in oxygen availability, other factors such as blood volume and other fluid concentrations could affect R_2* measures. To monitor this possible confounding factor, we checked that renal T1 values and kidney volume were consistent before and after placebo/GLP-1 interventions. Manually defined ROIs are widely reported but lead to an additional source of bias and variability, especially when related to the renal medulla. To reduce this bias, the perfusion and R_2* MRI data were coregistered, and the same ROIs were used for baseline and intervention time points.

Although careful standardization with respect to age, sex, body composition, and diet was attempted

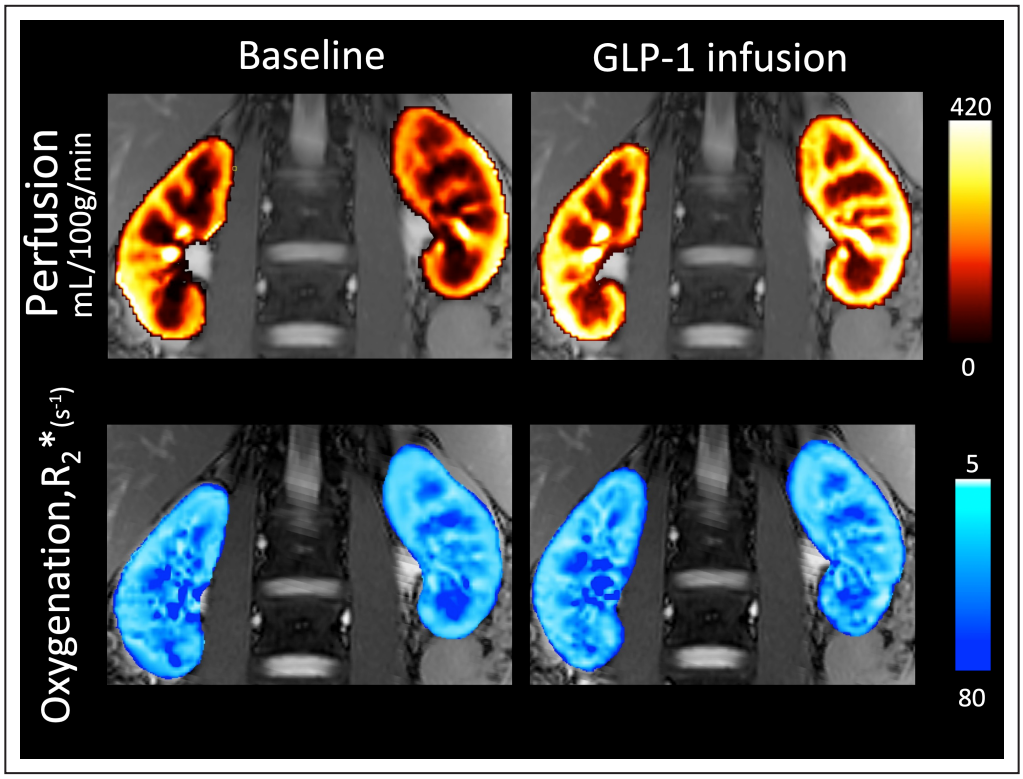

Figure 4. Perfusion and R_2^* maps for 1 participant immediately before (baseline) and 15 minutes after commencement of GLP-1 infusion.
Significant increases in both medullary and cortical perfusion were observed, as was either stable or slightly improved oxygenation (similar or reduced R_2^* values). Participants were in a steady state, having received saline infusion (750 mL/h) for 2 hours in the supine position before baseline scanning. GLP-1 infusion commenced in addition to the continued 750 mL/h saline infusion after baseline. GLP-1 indicates glucagon-like peptide-1; and R_2^*, oxygenation.

in the current study, the effect of acute elevation of plasma GLP-1 concentrations (slightly supraphysiological levels) in a limited number of healthy male participants poses clear limitations as to how much we can generalize, for example, in a larger population with type 2 diabetes treated with a long-acting GLP-1 receptor agonist and with women.

CONCLUSIONS

GLP-1 increases mainly renal medullary perfusion but also cortical perfusion and renal oxygen tension during NaCl loading. By preserving tissue oxygenation, improved perfusion may contribute to the long-term beneficial renal and cardiovascular effects of GLP-1 receptor agonists.

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