Glucagon-like peptide-1 does not have acute effects on central or renal hemodynamics in patients with type 2 diabetes without nephropathy

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Glucagon-like peptide-1 (GLP-1) does not have acute effects on central or renal hemodynamics in patients with type 2 diabetes without nephropathy. Glucagon-like peptide-1, a incretin hormone, stimulates insulin secretion and inhibits glucagon secretion. It is increased during feeding and is decreased by high-fat meals in patients with type 2 diabetes (1). Cardiac output increased, whereas renal hemodynamics remained unaffected. Despite marked venoarterial plasma concentration deficits of GLP-1 across the kidneys (~55%, henceforth designated renal extraction), no natriuretic effect could be demonstrated.

It is unknown whether native GLP-1 has the same effects in patients with type 2 diabetes. Thus, the present study was designed as a randomized, placebo-controlled, and single-blinded experiment with the purpose of investigating acute effects of exogenous GLP-1, resulting in plasma concentrations similar to those observed during conditions of accelerated gastric emptying (31) on central and renal hemodynamics in patients with type 2 diabetes without nephropathy.

METHODS

Patients. Baseline characteristics are shown in Table 1. Eight middle-aged male patients with type 2 diabetes participated in the study, which involved two experiments performed in random order and separated by ~4 wk. Mean duration of type 2 diabetes mellitus was 5.9 ± 1.4 yr, and mean Hb A1c was 7.3 ± 1.9%, 56 ± 17 mmol/mol. All patients were treated with metformin and statins only. None of the patients had microalbuminuria. Consent to participate was obtained after the patients had read a description of the experimental protocol, which was approved by the Scientific Ethics Committee of the Capital Region of Copenhagen (H-2-2013-099).

Protocol. For 4 days before each experiment, all patients consumed a controlled mixed diet (2,822 kcal/day; 16% protein, 55% carbohydrate, and 29% fat). The food was handed out frozen, and the basal sodium chloride content of the diet, measured at Eurofins Stein’s Laboratory in Denmark, was 55–75 mmol/day. Sodium chloride was added to the diet to standardize daily intake at 2 mmol sodium chloride·kg body wt−1·day−1. Twenty-four-hour urine was collected on the last day, and electrolyte, glucose, creatinine, and albumin concentrations were measured using Fick’s Principle after catheterization of a renal vein. Urine collection was conducted throughout the experiment.

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concentrations were determined. Water intake was ad libitum, and strenuous physical activity was not allowed. Patients were given 600 mg of lithium orally at 9 PM the day before each experiment. Under fixed sodium intake, it is generally accepted that the renal clearance and extraction of lithium correspond to the fractional sodium reabsorption in the proximal tubules (42).

Patients fasted for 12 h before the beginning of the experiments (Fig. 1). After emptying the bladder, confirmed by ultrasound, patients remained supine throughout the experiments. A forearm vein was catheterized with an 18-gauge catheter [BD Venflon: outer diameter (OD) 1.2 mm, length 45 mm; Becton-Dickinson, Helsingborg, Sweden] for infusions. The right femoral vein was catheterized using the Seldinger technique and a 6 F introducer (Avanti: OD 2.21 mm, length 110 mm; Cordis, Bridgewater, NJ). A 6 F catheter (Check-Flo Performer: OD 0.97 mm, length 750 mm; Cook Medical, Bloomington, IN) was advanced into the right or left renal vein under fluoroscopic control. The same vein was catheterized in both experiments. Since the oxygen saturation of renal venous blood is considerably higher than that of mixed blood in the inferior caval vein (41), we were able to check and confirm the position of the catheter tip in the renal vein throughout the experiments by measurements of oxygen saturation. A radial artery was catheterized with a 20-gauge catheter (BD Arterial Cannula: OD 1.1 mm, length 45 mm; Becton-Dickinson, Franklin Lakes, NJ) for blood sampling and for continuous monitoring of arterial blood pressure. Blood was collected simultaneously from the radial artery and the renal vein throughout the experiments, as described below. Renal plasma flow and glomerular filtration rate were measured via Wick’s Principle using chromium 51-labeled EDTA as indicator (51Cr-EDTA; GE Healthcare, Brøndby, Denmark). An intravenous bolus injection (2 MBq) in 10 ml of 0.9% NaCl was administered, followed by intravenous infusion (1.6 MBq/h for 6 h) in 0.9% NaCl (30 ml/h). Steady-state arterial concentrations of 51Cr-EDTA were obtained after ~90 min.

Blood pressure and heart rate were monitored invasively (ADInstruments, Oxford, UK). In addition, ECG was monitored throughout the experiments via a three-lead electrode applied to the chest. Measurements obtained during periods of ~5 min before and after blood sampling were used for analyses. After ≥2 h of infusion of 51Cr-EDTA, two baseline blood sample pairs were drawn, followed by the start of a 3-h infusion of either native GLP-1 (1.5 pmol·kg⁻¹·min⁻¹) or saline (0.9% NaCl) corresponding to a total volume of 60 ml and a NaCl amount of ~35 mmol. The solutions were prepared freshly. Patients were blinded with respect to the contents. During the experiments, bladder emptying was allowed with patients remaining in the supine position. At the end of the 3-h infusion, patients were instructed to perform deep breathing for 1 min with a frequency of 6 breaths/min to measure heart rate variability (35). This was repeated three times. A final bladder emptying was requested 2 h after the termination of infusion; the emptying was verified by ultrasound. The total amount of urine voided during each experiment was mixed and quantified.

Glucose clamp. In the first patient studied, arterial plasma glucose levels decreased by ~4 mmol/l during the GLP-1 infusion in accord with previous studies (44). The patient did not develop subjective symptoms of hypoglycemia, and post hoc analysis showed that arterial plasma adrenaline concentrations remained constant in this patient, indicating that a significant sympatico-adrenergic activation did not take place. Therefore, we did not exclude the patient from the study. Nevertheless, since one of the primary aims of the study was to elucidate direct central hemodynamic effects of GLP-1, we decided to clamp the plasma glucose concentrations at the fasting level (~8 mmol/l) in the remaining seven patients by infusion of glucose during the GLP-1 infusion to avoid any sympatico-adrenergic counterregulation due to hypoglycemia.

Materials. Synthetic human GLP-1 7–36amide was obtained from Bachem (Bubendorf, Switzerland) and 51Cr-EDTA from GE Healthcare.

Cardiac output. Estimated cardiac output was recorded continuously and noninvasively using Finapres (Finapres Medical Systems, Amsterdam, The Netherlands) (14). Measurements obtained in periods of ~5 min in length before and after blood sampling was used for analyses. The estimation of cardiac output via pulse contour analysis is an indirect method based on the development of the pulsatile unloading of the finger arterial walls using an inflatable finger cuff with built-in photoelectric plethysmograph (19, 20).

Blood and urine analyses. Samples of blood were drawn simultaneously from the radial artery and the renal vein every 20 min from time ~20 to 60 min and then every 60 min throughout the experiments. All arterial as well as venous blood samples were analyzed for GLP-1, glucose, sodium, lithium, hydrogen, potassium, hematocrit, oxygen saturation, and 51Cr activity. Insulin was analyzed only in arterial blood samples. Further, arterial blood samples drawn at baseline and 60, 120, and 180 min were analyzed for plasma adren-

### Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>58 ± 5</td>
</tr>
<tr>
<td>Height, cm</td>
<td>182 ± 7</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>94 ± 24</td>
</tr>
<tr>
<td>Fasting glucose concentration, mmol/l</td>
<td>7.9 ± 2.3</td>
</tr>
<tr>
<td>Fasting insulin concentration, pmol/l</td>
<td>87.4 ± 51.6</td>
</tr>
<tr>
<td>Hb A1c % (% mmol/mol)</td>
<td>7.3 ± 1.9 (56 ± 17)</td>
</tr>
<tr>
<td>Urinary albumin excretion, mg/24-h</td>
<td>7.1 ± 7.5</td>
</tr>
<tr>
<td>Urinary glucose excretion, mmol/24-h</td>
<td>15.2 ± 25.4</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD.
GLP-1 arterial. The extraction of GLP-1 was calculated as (GLP-1 arterial infusion-rate)/(51Cr-EDTA arterial excretions during baseline prior to GLP-1 or saline infusion) using a WIZARD3 1480 automatic gamma-counter (Perkin-Elmer, Waltham, MA). Each sample (0.5 ml) was counted for 60 min (10,000 counts).

Plasma was analyzed for gamma radiation activity on a automatic method (Cobas Integra 400; Roche Diagnostics). Albumin and glucose concentrations were measured using an enzymatic method (Cobas 8000 System; Roche Diagnostics, Indianapolis, IN). Urinary pH was measured using an XC161 Combination pH electrode (Radiometer Medical). Urinary electrolyte and lithium concentrations were measured using an enzymatic method (Cobas 8000 System; Roche Diagnostics). The tracer was prepared by in-house iodination and HPLC purification. Plasma samples were diluted 11-fold with RIA buffer prior to assay. Plasma levels of ANP were measured by radioimmunoassay using a commercial kit (2-CAT RIA; Labor Diagnostika Nord, Nordhorn, Germany). Plasma renin concentrations were determined using Liaison Direct Renin measurement (DiaSorin, Saluggia, Italy). Plasma aldosterone concentrations were determined by immunoassay using the Liaison autoanalyzer and kit from DiaSorin. Plasma levels of proANP were measured by radioimmunoassay (RIA) using antiserum and proANP(1–30) calibrator from Peninsula Laboratories. The tracer was prepared by in-house iodination and HPLC purification. Plasma samples were diluted 11-fold with RIA buffer prior to assay. Plasma levels of ANP were measured by enzyme immunoassay using a commercial kit (RayBio Human ANP Enzyme; RayBiotech, Norcross, GA). Blood samples for ANP analysis were collected in EDTA tubes prepared with 25 μl of 1,10-phenantrolin monohydrate. Urinary electrolyte and lithium concentrations were measured using an enzymatic method (Cobas 8000 System; Roche Diagnostics, Indianapolis, IN). Urinary pH was measured using an XC161 Combination pH electrode (Radiometer Medical). Urinary albumin and glucose concentrations were measured using an enzymatic method (Cobas Integra 400; Roche Diagnostics).

Calculations. Renal plasma flow was calculated as 51Cr-EDTA infusion-rate/(51Cr-EDTA arteria1l – 51Cr-EDTA venous) at steady state. Glomerular filtration rate was calculated as renal plasma flow × (51Cr-EDTA arteria1l – 51Cr-EDTA venous)/(51Cr-EDTA arteria1l). Renal extraction of GLP-1 was calculated as (GLP-1 arteria1l – GLP-1 venous)/GLP-1 arteria1l. Statistical analysis. The primary end point in the present study was the systolic blood pressure. When using a two-tailed α = 0.05 and requiring an 80% power threshold, the sample size n = 6 was calculated to detect appreciable effect of native GLP-1 on systolic blood pressure. This calculation was based on our previous study (1) in which the effect magnitude of a 3-h intravenous GLP-1 infusion on systolic blood pressure was 5 mmHg with an SD of change by 3 mmHg.

Data were analyzed using SigmaPlot 12 (Systat Software, Chicago, IL) and GraphPad Prism 5 (GraphPad Software, La Jolla, CA). Area under the curve (AUC) was calculated using the trapezoidal rule, and the t-test (2-tailed) for paired data was used for comparing ΔAUC during the GLP-1 infusion and ΔAUC during the saline infusion. Values of P < 0.05 were considered statistically significant.

RESULTS

Standardized sodium chloride intake. On the last day of the 4-day period with standardized sodium chloride intake prior to the GLP-1 or saline infusion, 24-h urine data were by and large equal (Table 2).

Renal uptake of GLP-1. During the GLP-1 infusion, arterial and renal venous plasma concentrations of total GLP-1 in-

Table 2. Twenty-four-hour renal fluid and electrolyte excretions during baseline prior to GLP-1 or saline infusion

<table>
<thead>
<tr>
<th>24-h Urine Variable</th>
<th>GLP-1</th>
<th>Saline</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume, ml</td>
<td>2,560 ± 372</td>
<td>2,571 ± 379</td>
<td>0.977</td>
</tr>
<tr>
<td>Sodium, mmol</td>
<td>220 ± 22</td>
<td>213 ± 27</td>
<td>0.872</td>
</tr>
<tr>
<td>Lithium, mmol</td>
<td>3.6 ± 0.7</td>
<td>4.3 ± 1.4</td>
<td>0.681</td>
</tr>
<tr>
<td>Hydrogen, mmol</td>
<td>4,672 ± 984</td>
<td>3,827 ± 1238</td>
<td>0.611</td>
</tr>
<tr>
<td>Potassium, mmol</td>
<td>108 ± 32</td>
<td>109 ± 13</td>
<td>0.974</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE. GLP-1, glucagon-like peptide-1.
creased significantly to 126 ± 7 and 79 ± 12 pmol/l, respectively, and reached a steady state after 20 min (Fig. 2A).

Analysis of the separate contributions of intact GLP-1 7–36amide and the metabolite GLP-1 9–36amide demonstrated that both moieties were extracted (~50%, P < 0.001, and ~30%, P < 0.001, respectively; Fig. 2, B and C). During the saline infusion, arterial and renal venous plasma concentrations of total GLP-1 remained constant throughout the experiments (5 ± 1 and 4 ± 1 pmol/l), and a significant renal extraction of GLP-1 could not be demonstrated (Fig. 2A).

Effects of GLP-1 on arterial plasma levels of insulin, glucose, and vasoactive hormones. Plasma glucose concentrations were clamped at the fasting level (~7.0–8.0 mmol/l) throughout the GLP-1 infusion. This demanded an average glucose infusion of 28.6 ± 3.8 g of a 20% glucose solution (Fig. 3, C and D). On the other hand, it led to a sustained increase in arterial plasma insulin levels throughout the infusion (~3-fold increase, P = 0.014) (Fig. 3, A and B). During the saline infusion, plasma glucose levels remained constant, rendering clamping superfluous (Fig. 3, C and D). Arterial plasma levels of vasoactive hormones remained unchanged during the experiments (Fig. 4, A–L).

Effects of GLP-1 on heart rate and heart rate variability. Heart rate increased immediately and remained elevated throughout the GLP-1 infusion (P < 0.001; Fig. 5, G and H), whereas mean heart rate range (heart rate variability) was normal and similar during the two experiments [GLP-1: 22 ± 4 beats/min (33%); saline: 21 ± 2 beats/min (33%)] (P = 0.888).

Effects of GLP-1 on blood pressure, cardiac output, and renal hemodynamics. During the GLP-1 infusion, systolic (Fig. 5, A and B) and diastolic (Fig. 5, C and D) blood pressure tended to increase initially but insignificantly compared with the saline infusion. Cardiac output remained unchanged in both experiments (Fig. 5, I and J). RPF and glomerular filtration rate (GFR) were unaffected during the experiments (Fig. 6, A and D), and the filtration fraction was normal (20 ± 2%). Mean individual levels of RPF and GFR throughout the GLP-1 or saline infusion are shown in Fig. 7, A and B.

Renal arterio-venous plasma electrolyte concentrations and electrolyte and glucose excretions. Differences in arterio-venous plasma concentrations of sodium, potassium, hydrogen, and lithium could not be demonstrated (data not shown). Furthermore, renal excretion of these substances remained unchanged during the GLP-1 compared with saline infusion (Fig. 7, C–H).

**DISCUSSION**

The major finding in the present study is the sustained positive chronotropic effect on the heart during acute administration of native GLP-1 at a rate resulting in plasma concentrations similar to those observed during conditions of accelerated gastric emptying, gastric bypass operations, or large fat- and protein-containing meals (31). Cardiac output remained constant, and despite renal uptake of GLP-1 well in excess of the glomerular filtration, it was not possible to demonstrate any effect on RPF, GFR, or sodium excretion. In addition, a GLP-1-ANP axis could not be demonstrated.

**GLP-1 and blood pressure.** To our knowledge, this is the first human study designed specifically to investigate the acute effects of native GLP-1 on blood pressure, measured invasively, in patients with type 2 diabetes. In the present study, we demonstrate that systolic and diastolic blood pressure levels remained mainly unchanged during the GLP-1 or saline infusion. This is in contrast to our recent study (1) conducted in healthy individuals, in whom systolic blood pressure increased.

![Graphs](image-url)
during the GLP-1 infusion, whereas diastolic blood pressure remained unchanged, indicating increased stroke volume.

**Cardiac chronotropic effect of GLP-1.** A sustained positive chronotropic effect was seen during the GLP-1 infusion as also seen in healthy individuals in our recent study (1). The GLP-1 infusions also brought an approximately threefold increase in arterial plasma insulin concentrations maintained throughout the GLP-1 infusion because plasma glucose concentrations were clamped at the patients’ fasting glucose level, ~8 mmol/l. In healthy individuals, euglycemic hyperinsulinemia at high physiological/pharmacological concentrations (~400 pmol/l, ~10-fold increase) was accompanied by sympathoexcitatory effects (a physiological effect determined by elevations in plasma noradrenaline concentrations and increased muscle sympathetic nerve activity) presumably elicited via central neural action and baroreflex activation secondary to peripheral vasodilation in the skeletal muscle tissue (30, 33, 41, 45), possibly also induced by insulin (a pharmacological effect) (34), without affecting blood pressure (47). In the present study, plasma catecholamine concentrations were unaffected. Thus, it seems reasonable to conclude that the degree of hyperinsulinemia in the present study most likely did not affect sympathetic nervous activity. Alternatively, the increase in heart rate may be due to activation of GLP-1 receptors (GLP-1Rs) located perhaps in the sinoatrial node (32).

**Pulse pressure and aging.** In the present study, patients with type 2 diabetes were significantly older compared with the healthy individuals (58 ± 2 vs. 46 ± 2 yr) in our previous study (1). Therefore, an age-dependent general stiffening of the arterial system may explain that the arterial pulse pressure was higher in the patients with type 2 diabetes (77 ± 5 vs. 61 ± 3 mmHg). Cardiac output did not differ between the GLP-1 or saline infusion. To obtain accurate absolute measurements of stroke volumes and cardiac output levels, a calibration of the Finapres method against a direct method such as the Fick’s Principle (e.g., indicator dilution) is required. However, such calibration is not necessary for registration of possible relative changes in cardiac output in the present study due to the GLP-1 infusion (4, 40).

**The endothelium and vascular tonus.** Several studies provide evidence for impaired endothelium-dependent and -independent vasoactive responses to acetylcholine in patients with type 2 diabetes (22). In previous human studies (24, 27), GLP-1 administration was shown to improve this endothelial dysfunction, whereas the effects during chronic incretin-based therapy, assessed by flow-mediated dilation (FMD), are contradictory (29). However, most randomized prospective clinical studies demonstrate neutral FMD responses (12, 16, 25).

In healthy individuals, we demonstrated that GLP-1 increased cardiac output (~18%, 1.2 ± 0.1 l/min) to a larger degree than the increase in mean arterial pressure (~2%, 2.9 ± 1.4 mmHg) (1). This indicates that a peripheral vasodilation took place. However, renal hemodynamics were not affected. In another randomized, placebo-controlled crossover study (24), hemodynamics were investigated in healthy male individuals 2 h after a single 10-μg subcutaneous injection of exenatide. Cardiac output, assessed by the Finapres device, increased by a mean of 1.2 l/min (95% confidence interval, 0.42, 2.03). Furthermore, leg blood flow (FMD response) increased. This suggests that the increase in cardiac output was compensatory to vasodilation in other beds than in the kidneys,
e.g., in the skeletal muscles, and/or adipose tissue. In our study, an impaired vascular function may explain the absence of a GLP-1-induced increase in cardiac output in patients with type 2 diabetes.

**Insulin and central hemodynamics.** It has been demonstrated previously in clamp experiments conducted in healthy subjects that euglycemic hyperinsulinemia at high physiological/pharmacological insulin concentrations (400–600 pmol/l, 10-fold increase) evoked increased sympathetic tone via central mechanisms (a physiological effect) and also vasodilation in the skeletal muscle tissue (a pharmacological effect) (45–47). In obese subjects with normal glucose tolerance (46), even higher levels of insulin did not have any detectable effect on either muscle sympathetic nerve activity or vascular resistance. This may represent another feature of insulin resistance in obesity in which chronic hyperinsulinemia precludes the demonstration of any additional sympathoexcitation during acute increases in plasma insulin levels, as seen during the GLP-1 infusion.

Fig. 5. Intra-arterial blood pressure (A–F), heart rate (G and H), and cardiac output (I and J). A, C, E, G, and I: time course of the measurements during the infusions from 0 to 180 min. B, D, F, H, and J: integrated effect during the infusions from 0 to 180 min compared with baseline levels. Data are presented as means ± SE.
In the present study, plasma insulin levels increased approximately threefold throughout the GLP-1 infusion, but cardiac output and plasma noradrenaline levels remained unchanged, indicating that an insulin-mediated skeletal muscle vasodilation and/or central activation of the sympathetic nervous system did not take place. This would be compatible with the fact that our subjects were insulin resistant. 

Renal uptake of GLP-1. Like in healthy individuals, GLP-1 is taken up in the kidneys in patients with type 2 diabetes without nephropathy. The large arterio-venous GLP-1 concentration difference exceeds what can be explained by glomerular filtration. In this context, it is of interest that the GLP-1R has been localized to the renal afferent arterioles, and binding to these with subsequent internalization could be responsible for the additional clearance (32). In a recent human study (38), urinary levels of GLP-1 remained at the detection limit of the assay during a 2-h GLP-1 infusion. Thus, mechanisms other than urinary excretion of GLP-1 must explain the renal handling of GLP-1, e.g., GLP-1-GLP-1R internalization. Effects of the GLP-1-degrading enzyme dipeptidyl peptidase-4 (DPP-4) in the renal vasculature cannot explain the concentration difference, since this was also seen for total GLP-1 concentrations, which are not affected by DPP-4 degradation, but DPP-4 degradation may contribute since the extraction ratio was slightly higher for intact compared with total GLP-1. Despite the significant renal uptake of GLP-1, which suggests that GLP-1 is bound to and possibly activated GLP-1Rs, we were not able to measure any change in renal sodium, lithium, hydrogen, or potassium excretion during the GLP-1 infusion, in agreement with constant renal hemodynamics, indicating that GLP-1 under the applied experimental conditions does not have significant physiological effects on renal function.

GLP-1 and natriuresis. In contrast to the present study, previous human studies (8, 38) demonstrated remarkable GLP-1-induced acute natriuresis (~40–60%) in overhydrated individuals, suggesting that GLP-1 may contribute to natriuresis via volume regulating mechanisms (3). Because of a simultaneous decrease in renal hydrogen excretion, Gutzwiller et al. (8) suggested that GLP-1 decreases proximal tubular sodium reabsorption by inhibiting the NHE3-mediated Na/H exchange. This was supported by the study of Skov et al. (38), in which GLP-1 increased renal sodium clearance concomitantly with an increase in renal lithium clearance. However, expression of GLP-1Rs could not be demonstrated in human proximal tubular cells (18, 32).

It must be emphasized that the mode of urine collection conducted in the present study did not allow any time resolution with respect to a possible transient natriuretic effect of GLP-1. Nevertheless, we recently calculated (1) that a detectable renal arterio-venous difference in plasma sodium concentrations should be measurable if the previously published 40–60% increase in natriuresis by GLP-1 was taking place (8, 38).

In mice, GLP-1R activation was reported to stimulate ANP secretion from atrial cardiomyocytes, leading to natriuresis and vasodilation (17). However, a GLP-1-ANP axis most likely does not exist in man either in patients with type 2 diabetes or in healthy individuals (1, 21).

Insulin and sodium retention. Hyperinsulinemia (680-1,340 pmol/l) causes substantial sodium retention (~50%), an effect that is preserved in insulin-resistant individuals (5, 13). Thus, the glucose clamp conducted in the present study during the GLP infusion is a potentially significant limitation due to increased plasma insulin levels. Sodium retention induced by hyperinsulinemia is independent of changes in renal hemodynamics (5). In a previous study (6), urinary sodium excretion in

Fig. 6. Renal plasma flow (A and B) and glomerular filtration rate (C and D). A and C: time course during the infusions from 0 to 180 min. B and D: integrated effect during the infusions from 0 to 180 min compared with baseline levels. Data are presented as means ± SE.
response to an oral glucose tolerance test was compared between obese and nonobese subjects. The endogenous hyperinsulinemia due to an oral glucose tolerance test was, as expected, significantly higher (~3-fold) in obese (950 ± 150 pmol/l) compared with nonobese subjects (330 ± 55 pmol/l). Baseline urinary sodium excretion was similar between obese and nonobese subjects; however, after the glucose tolerance test, urinary sodium excretion decreased only in obese subjects, and the decrease was strongly correlated to plasma insulin levels. Thus, sodium retention was not detectable with insulin concentrations comparable with those found in the present study. Because we were not able to demonstrate any renal effects of GLP-1 in our previous study (1), we find it unlikely that the similar lack of effects in the present study should be explained by opposing effects of GLP-1 and hyperinsulinemia, outweighing each other.

GLP-1 and renal hemodynamics. This is the first study to investigate acute renal hemodynamic effects of native GLP-1 in patients with type 2 diabetes, and similarly to our previous study in healthy individuals, RPF and GFR were unaffected by GLP-1. This is in accord with previous findings in healthy individuals (38). However, it is possible that the effect of GLP-1 on GFR may be different in kidneys with glomerular hyperfiltration. In obese, insulin-resistant males with hyperfiltration, GFR decreased by ~6% (from 151 ± 8 to 142 ± 8 ml/min) during a 3-h GLP infusion (8), whereas GFR remained unchanged in healthy lean males. GFR was determined by endogenous creatinine clearance, which is known to be inaccurate (39). In lieu of renal inulin clearance (the “gold standard”), renal clearance of $^{51}$Cr-EDTA is suggested as an accurate method. However, in non-steady-state conditions, GFR may be underestimated due to a small amount of $^{51}$Cr-EDTA being plasma protein bound (39). In the present study, the arterial $^{51}$Cr-EDTA levels were constant (steady state) during the experiments. Thus, we consider the present measurements accurate for RPF and GFR and the method suffi-
ciently sensitive for detection of possible changes of biological importance.

Limitations of the study. The approximately threefold increased plasma insulin levels induced by the GLP-1 infusion during the euglycemic glucose clamp is a potential limitation, since it cannot be completely excluded that it may have led to an increased renal sodium reabsorption counterbalancing a possible GLP-1-induced natriuresis. Furthermore, the effect of acute elevation of plasma GLP-1 concentrations for a few hours in a limited number of patients with type 2 diabetes, given that these patients are both genotypically and phenotypically heterogeneous, may not necessarily reflect the chronic elevation of GLP-1R agonist levels in a larger population with type 2 diabetes treated with a long-acting GLP-1R agonist. Therefore, the existence of chronic effects that are not detected in the present experimental setup cannot be excluded.

Conclusions. Under the controlled conditions applied in the present experiments, GLP-1 increases heart rate but does not increase cardiac output in patients with type 2 diabetes. Renal hemodynamics and sodium excretion are not affected. A GLP-1-ANP axis most likely does not exist in patients with type 2 diabetes.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES
