Liraglutide Promotes Natriuresis but Does Not Increase Circulating Levels of Atrial Natriuretic Peptide in Hypertensive Subjects With Type 2 Diabetes

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OBJECTIVE

GLP-1 receptor (GLP-1R) agonists induce natriuresis and reduce blood pressure (BP) through incompletely understood mechanisms. We examined the effects of acute and 21-day administration of liraglutide on plasma atrial natriuretic peptide (ANP), urinary sodium excretion, office and 24-h BP, and heart rate (HR).

RESEARCH DESIGN AND METHODS

Liraglutide or placebo was administered for 3 weeks to hypertensive subjects with type 2 diabetes in a double-blinded, randomized, placebo-controlled crossover clinical trial in the ambulatory setting. End points included within-subject change from baseline in plasma ANP, Nt-proBNP, office BP, and HR at baseline and over 4 h following a single dose of liraglutide (0.6 mg), and after 21 days of liraglutide (titrated to 1.8 mg) versus placebo administration. Simultaneous 24-h ambulatory BP and HR monitoring and 24-h urine collections were measured at baseline and following 21 days of treatment.

RESULTS

Plasma ANP levels did not change significantly after acute (+16.72 pg/mL, $P = 0.24$, 95% CI [−12.1, +45.5] at 2 h) or chronic (−17.42 pg/mL, 95% CI [−36.0, +1.21] at 2 h) liraglutide administration. Liraglutide significantly increased 24-h and nighttime urinary sodium excretion; however, 24-h systolic BP was not significantly different. Small but significant increases in 24-h and nighttime diastolic BP and HR were observed with liraglutide. Body weight, HbA$_{1c}$, and cholesterol were lower, and office-measured HR was transiently increased (for up to 4 h) with liraglutide administration.

CONCLUSIONS

Sustained liraglutide administration for 3 weeks increases urinary sodium excretion independent of changes in ANP or BP in overweight and obese hypertensive patients with type 2 diabetes.

Patients with type 2 diabetes experience a greater than twofold excess risk for the development of cardiovascular disease (1,2), with coexistent hypertension further increasing the risk of cardiovascular complications (3). Even modest reductions in blood pressure (BP) reduce the risk of stroke and myocardial infarction (4).

Accordingly, there is great interest in therapies that might not only improve glucose but also enhance BP control in hypertensive diabetic subjects.

Among various classes of antidiabetic agents, both sodium-glucose cotransporter-2 (SGLT2) inhibitors and GLP-1 receptor (GLP-1R) agonists are associated with further reduction of BP in hypertensive subjects. Although the antihypertensive actions of SGLT2 inhibitors are thought to be largely mediated through enhanced urinary sodium excretion and osmotic diuresis (5), the mechanisms through which GLP-1R agonists reduce BP are less well understood and may include weight loss, direct or indirect vasorelaxation, or stimulation of natriuresis (6).

Both SGLT2 inhibitors and GLP-1R agonists exert their glycemic effects through mechanisms that are glucose dependent, resulting in low rates of hypoglycemia. Similarly, both drug classes reduce BP in hypertensive subjects through mechanisms that are attenuated in normotensive subjects, thereby diminishing the likelihood of treatment-associated hypotension. Interestingly, natriuresis is induced by both SGLT2 inhibitors and GLP-1R agonists through different mechanisms; notably, the pathways and mechanisms linking GLP-1R signaling to renal sodium excretion are controversial and less well understood (7). Although short-term infusion (3 h) of GLP-1$^{7\text{-}36amide}$ increases natriuresis in healthy volunteers (8,9) and in insulin-resistant obese males (8), whether these actions are sustained with chronic GLP-1R activation is unclear. Furthermore, the majority of studies examining how GLP-1R agonists increase sodium excretion are often 3–72 h in duration (7–11). Hence, there is limited information on how sustained administration of GLP-1R agonists regulates mechanisms leading to BP reduction in hypertensive subjects with type 2 diabetes.

We recently examined pathways linking GLP-1R signaling to control of BP in nondiabetic hypertensive mice. These studies demonstrated that structurally diverse GLP-1R agonists, including native GLP-1, liraglutide, and exenatide, increased plasma levels of atrial natriuretic peptide (ANP), enhanced natriuresis, and reduced BP in mice with angiotensin II–induced hypertension (12). In this context, we examined whether liraglutide increased ANP and urinary sodium excretion in a prospective, double-blinded, randomized, placebo-controlled, crossover trial in 20 hypertensive patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS

Study Design

This study was a single-center, prospective, double-blinded, randomized (1:1), placebo-controlled, crossover study that took place at Mount Sinai Hospital, Toronto, Ontario, Canada. Subjects were randomized to liraglutide or to placebo for 21 days during two separate treatment periods (treatment period 1 and treatment period 2) separated by a 21-day treatment washout period (Supplementary Fig. 1A and B). Ten patients started in treatment sequence A (first randomized to receive liraglutide), and 10 patients were randomized to start in treatment sequence B (first randomized to receive placebo). The study coordinators enrolled patients and pharmacy-assigned participants to interventions according to the randomization sequence that was computer generated. Treatment allocation was blinded to patients and study personnel until the database was locked for analysis. Recruitment commenced January 2013 and ended October 2013, and follow-up ended January 2014.

During treatment period 1, subjects self-administered study drug (liraglutide or placebo) daily, at an initial dose of 0.6 mg for the 1st week (day 1, clinic visit 1) and then 1.2 mg for the 2nd week followed by 1.8 mg until day 21 (clinic visit 2) (Supplementary Fig. 1A). To preserve blinding, placebo was volume matched, with a two-step sham titration, and delivered in a pen device identical to that used for liraglutide. After completion of the first 21-day treatment period, subjects underwent a 21-day washout period and started treatment period 2 (crossover to opposite treatment sequence) on day 42 (clinic visit 3). All subjects continued their routine medications throughout the study; however, if a patient was treated with a sulphonylurea prior to study entry, the dose of the sulphonylurea was reduced by 50% at the start and for the duration of each treatment phase. Antihypertensive medications were temporarily discontinued for 1–2 days prior to each clinical visit. One patient could not tolerate temporary discontinuation of their antihypertensive medications and completed test days on therapy, and two patients neglected to withhold their antihypertensive medications at clinic visit 1. To maintain subject consistency between visits, they were instructed to complete the remaining clinic visits and tests on therapy. All of these patients were included in the study population for the analysis.

Study Population

Study entry criteria included subjects with type 2 diabetes with an HbA$_{1c}$ ≥6.5 and ≤10.1%, with systolic hypertension (systolic BP [SBP] ≥130 and ≤180 mmHg) and aged >18 years. Exclusion criteria reflected conditions contraindicated with the recommended use of liraglutide (http://www.pbm.va.gov/clinicalguidance/drugmonographs/Liraglutidemonograph.pdf). Recruitment occurred directly from family physician or specialty clinics or via self-referral in response to advertising as summarized in Supplementary Fig. 2.

Experimental Protocol

The study protocol was approved by the Mount Sinai Hospital Research Ethics Board (Toronto, Ontario, Canada) and performed in accordance with the Declaration of Helsinki and the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans. This study was registered at www.clinicaltrials.gov (NCT01755572).

Baseline Tests

After study eligibility criteria were confirmed (Supplementary Fig. 2), subjects entered the baseline test period, which consisted of a 24-h ambulatory blood pressure monitoring (ABPM) and a 24-h urine collection (1–7 days prior to day 1) as described below.

Clinic Visits

Subjects attended four clinic visits (days 1, 21, 42, and 63) at the start and end of each treatment period (Supplementary Fig. 1A) and were instructed to withhold antihypertensive medications (24–48 h) and oral antidiabetic agents (12 h) before clinic visits. Patients fasted prior to each assessment (12 h) and were instructed to avoid caffeine, smoking, and exercise on the day before and morning of all clinic visits. Office BP and heart rate (HR) were measured

serially (average of five readings over 6 min, first reading discarded) by a calibrated, automated oscillometric sphygmomanometer (BpTru; BpTru Medical Devices, Coquitlam, British Columbia, Canada). These measurements were performed prior to study drug administration (time = 0 h) and at the end of the clinic visit (time = 4 h).

Blood Collection

A venous cannula was inserted in the subject's forearm and blood was sampled at −5 min prior to study drug administration and then hourly for 4 h thereafter (Supplementary Fig. 1B). Blood was collected at room temperature or, for ANP and angiotensin II collection, into prechilled (−4°C) 4-mL K_2EDTA tubes to which 50 μL of aprotinin (A6279; Sigma-Aldrich [for ANP]) or 100 μL bestatin (002-A22/B-BST ALPCO; Fisher Scientific [for angiotensin II]) had been added. Samples were either immediately centrifuged (1,800g) for 10 min at 4°C, aliquoted, and stored (−80°C) or sent to the clinical laboratory for biochemical analysis.

Biochemical Assays

Plasma ANP concentrations were measured in duplicate using an EIA kit (protocol III, catalog no. S-1131; Peninsula Laboratories, Inc., Bachem International). Angiotensin II concentrations were determined using an RIA kit (Buhlmann Laboratories, ALPCO Diagnostics, Salem, NH). All other analytes were measured in the clinical laboratories of Mount Sinai Hospital.

Ambulatory BP (24 h)

Ambulatory BP (24 h) was assessed using a Spacelabs system (model 90207–30) at baseline and 1–2 days prior to the end of each treatment period (days 21 and 63). Subjects recorded their sleep and awake times over 24 h. The ambulatory BP readings (Cardiology Information Management System, version 9.0.2.4475; Sentinel, Spacelabs Healthcare) were interpreted by a nephrologist blinded to treatment allocation. One individual had incomplete 24-h ABPM recordings; hence, in addition to the 2 subjects excluded due to significant protocol deviations (Supplementary Fig. 2), the number of individuals included in the BP analysis was 17.

Urine Collections (24 h)

Urine collections were performed simultaneously with the 24-h ABPM 1–2 days prior to clinic visits. Patients did not undergo sodium restriction; however, they were instructed to maintain a consistent dietary intake throughout the study.

Statistical Methods

Independent statisticians performed the statistical analyses. The primary outcome was the within-subject hourly and baseline change in plasma ANP between liraglutide or placebo assessed after the first injection or after 3 weeks of daily administration. Secondary outcomes included within-subject differences in 24-h ABPM and 24-h urine sodium excretion after 3 weeks of treatment between liraglutide and placebo. For the primary outcome, a sample size of 16 participants would provide 80% power to detect a 20% difference in plasma ANP at a significance level (α) of 0.05. This calculation was based upon the estimated treatment effect observed in preclinical studies for plasma ANP and liraglutide (12). Supplementary Table 1 provides descriptive statistics for patient baseline characteristics where the mean and standard deviation are reported for continuous variables and the median with interquartile range are presented for skewed variables. For categorical variables, the counts and percentages are provided.

The treatment effect between the two interventions, liraglutide and placebo, was estimated from a linear random-effects model with a random effect for subject and fixed effects for period, sequence, and treatment. For the linear regression models, residual diagnostics were performed to check for the validity of the models. To determine the treatment significance for nonnormal distributed data, nonparametric Wilcoxon signed rank test was used.

For secondary end points (24-h ABPM and urine sodium), the within-subject differences were calculated between liraglutide and placebo following 21 days of treatment as compared with baseline measurements. All statistical analyses were performed using Statistical Analysis System (SAS, version 9.1; SAS Institute, Cary, NC) with two-sided probability values <0.05 considered to be statistically significant.

RESULTS

Subjects

Eighteen participants were included in the analysis (2 subjects were excluded due to significant protocol deviations), with the exception of the 24-h ABPM for which 17 individuals were included (1 subject excluded due to incomplete 24-h ABPM tests). The study population primarily consisted of overweight and obese (BMI = 29.50 kg/m^2) males, median age 62 years, with a history of type 2 diabetes of 5.8 years and a mean baseline HbA$_{1c}$ of 7% (Supplementary Table 1). The majority of subjects were treated with metformin (95%) and/or a sulfonylurea (35%). At screening, the median SBP and diastolic BP (DBP) was 144 and 85 mmHg, respectively; 58% of subjects were nondippers (failure to reduce nocturnal SBP by 10% relative to day) and 18% were risers (increase in nocturnal SBP relative to day). Surprisingly, 44% of the study population was not being treated for hypertension. The median ACR was 1.38 mg/mmol, and several individuals had microalbuminuria (>30 mg/24 h).

Cardiac Natriuretic Hormones

Plasma ANP levels were unchanged after the first single 0.6-mg injection of liraglutide (+16.72 pg/mL, P = 0.24, 95% CI [−12.1, 45.5] at 2 h) (Fig. 1A and Supplementary Fig. 3A). Similarly, after 21 days of liraglutide (1.8 mg) therapy (Fig. 1B and Supplementary Fig. 3B), no statistically significant changes were observed in plasma ANP levels with liraglutide compared with placebo (−17.42 pg/mL, P = 0.07, 95% CI [−36.0, +1.21] at 2 h). No significant changes in serum Nt-proBNP concentrations were observed at day 1 or following 21 days (Supplementary Fig. 3C and D).

Urine Sodium

A significant increase in 24-h urinary sodium excretion (median change 14.18 mmol/L liraglutide vs. placebo) (Fig. 2) and nighttime urinary sodium excretion (median change 4.24 mmol/L nighttime, liraglutide vs. placebo) was observed following 21 days of liraglutide (Fig. 2A and B). No significant changes in the albumin-to-creatinine ratio were observed with liraglutide (Supplementary Fig. 4A and B).

BP and HR

No differences were observed in 24-h, daytime, or nighttime (Table 1) or office SBP (Supplementary Table 3). Although liraglutide did not produce significant reductions in SBP, a robust drop in SBP for liraglutide was observed between
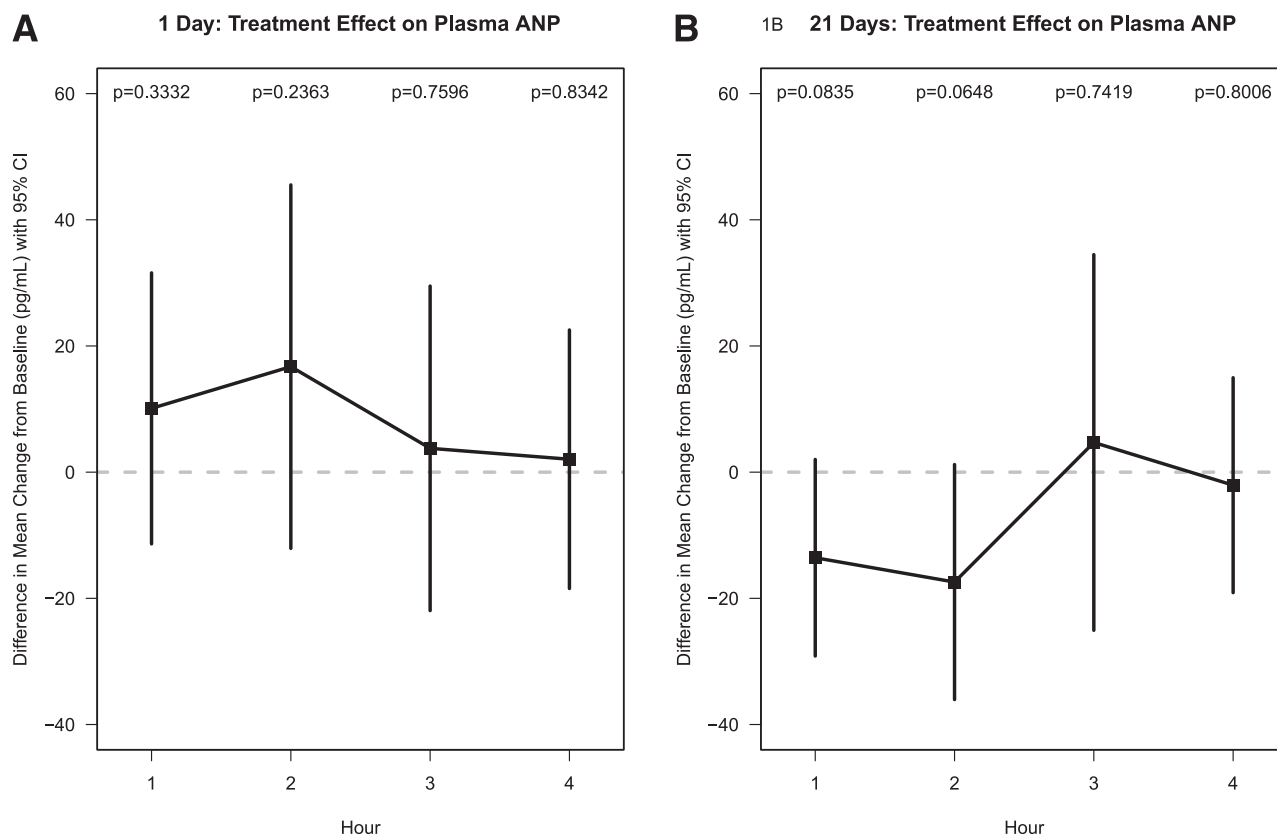
A   **1 Day: Treatment Effect on Plasma ANP**   **B**   1B   **21 Days: Treatment Effect on Plasma ANP**

Figure 1—Treatment effect of 1- or 21-day liraglutide treatment on cardiac natriuretic peptides compared with crossover treatment with placebo. The difference in mean change from baseline in plasma ANP concentrations was measured hourly following 1-day (*A*) and following 21-day (*B*) treatment with liraglutide and following crossover treatment with placebo. The data are presented as the hourly mean difference from baseline with 95% CI. Treatment doses: 1 day (0.6 mg, single dose) and 21 day (1.8 mg, final dose).

2100 h and 0200 h (Fig. 3). Small, yet statistically significant, increases in 24-h (least squares mean difference [LSMD] +3.78 $\pm$ 2.81 mmHg, *P* = 0.01) and nocturnal DBP (LSMD +3.77 $\pm$ 1.74 mmHg, *P* = 0.05) were observed (Table 1) with liraglutide treatment as compared with placebo. The within-subject difference for 24-h (LSMD +5.21 $\pm$ 2.42, *P* = 0.05) and nighttime HR (LSMD +7.34 $\pm$ 2.38, *P* = 0.008) was statistically increased with liraglutide treatment compared with placebo. A significant rise in baseline (time = 0 h) HR measured in the office on clinic days was observed after 21 days of liraglutide (LMSD, +9.25 $\pm$ 3.5 bpm, *P* = 0.02) (Supplementary Table 3); however, by 4 h after liraglutide injection, the within-subject increases in office-measured HR were no longer statistically significant (LSMD +3.69 $\pm$ 3.02 bpm, *P* = 0.24) (Supplementary Table 3).

Metabolic End Points
Liraglutide significantly reduced levels of HbA$_{1c}$ (mean 7.04–6.61%, LSMD −0.7%, *P* = 0.005), fasting plasma glucose (8.39 to 5.58 mmol/L, LSMD −3.4 mmol/L, *P* = 0.0004), and cholesterol (mean 4.16 to 3.64 mmol/L, LSMD −0.63 mmol/L, *P* = 0.002 for total cholesterol; 2.00 to 1.78 mmol/L, LSMD −0.37 mmol/L, *P* = 0.04 for LDL cholesterol) (Supplementary Table 2). No significant changes in serum creatinine or plasma angiotensin II concentrations were detected. There was a statistically significant decrease in estimated glomerular filtration rate (Supplementary Table 2). Liraglutide reduced body weight (LSMD −1.35 $\pm$ 0.46 kg, *P* = 0.009) and BMI (LSMD −0.43 $\pm$ 0.18 kg/m^2, *P* = 0.03), without changes in waist circumference (Supplementary Table 3).

Adverse Events
All 20 patients completed the research study, and no serious adverse events occurred (Supplementary Table 4). The most common complaints were a loss of appetite (30%) or were gastrointestinal in nature, including nausea (20%), dyspepsia (15%), and flatulence (15%).

One individual was unable to tolerate dose titration due to dyspepsia and completed the study at the lower dose (1.2 mg) following retitration.

CONCLUSIONS
A gut-cardiac GLP-1R–ANP axis was recently identified in nondiabetic rodents with angiotensin II–induced hypertension (12); however, the current findings do not suggest a role for a GLP-1R–ANP axis in transducing the renovascular effects of liraglutide in hypertensive subjects with type 2 diabetes. Our data demonstrating no acute increase in plasma ANP levels after liraglutide administration are consistent with results from a study of native GLP-1 (1.25 pmol/kg) in 12 healthy, young, normal-weight, male human subjects. Although a 2-h infusion of GLP-1 increased urinary sodium excretion (11), there were no changes in levels of circulating pro-ANP over 2 h.

Our current findings extend existing knowledge of the relationships encompassing GLP-1 action and changes
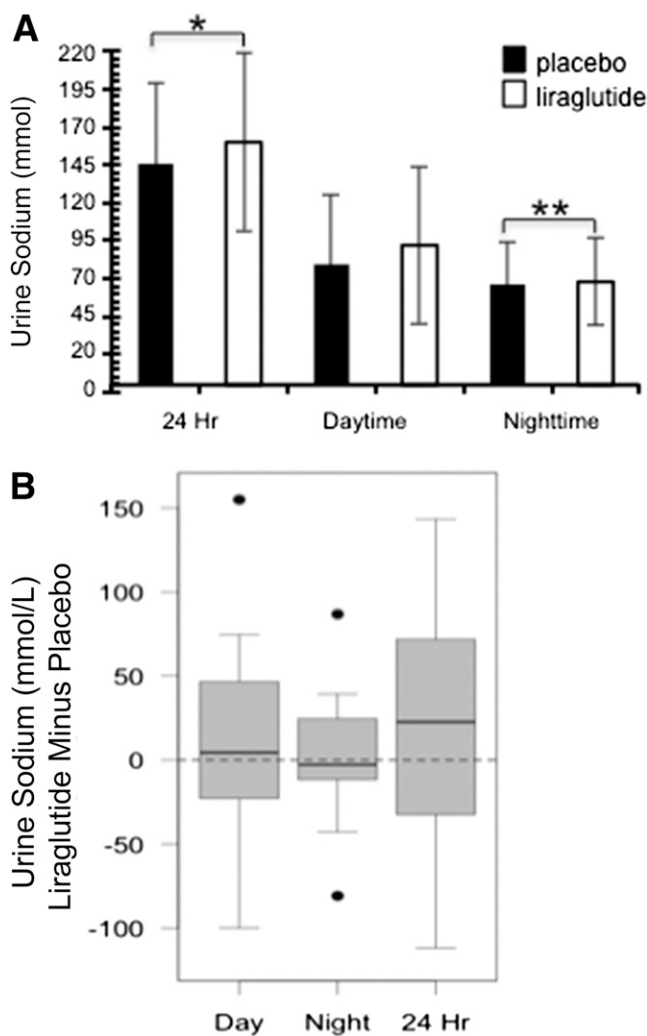
Figure 2—The effect of 21-day treatment with liraglutide on 24-h, daytime, and nighttime urinary sodium excretion compared with crossover treatment with placebo. *A*: Median (95% CI) urine sodium for 24 h, daytime, and nighttime for the treatment groups (liraglutide and placebo). *B*: The within-subject median change in urine sodium excretion for liraglutide minus placebo is presented in the box plot and whiskers graph for change. For the box plots and whiskers graph, the horizontal line indicates the median change, the box represents interquartile range of the change, and outliers are presented as single points. Nonparametric tests were used for comparison (Wilcoxon rank sum). *, the normal approximation for two-sided *P* value <0.05; **, the normal approximation for two-sided *P* value <0.005 for liraglutide compared with placebo.

in cardiorenal parameters in several ways. First, we did not restrict measurement of natriuretic peptides to a single time point; rather, we assessed serial changes in plasma levels of ANP and Nt-proBNP over time, in acute studies, and after 21 days of liraglutide administration. Second, we simultaneously assessed whether sustained liraglutide therapy was associated with changes in urinary sodium excretion. Third, our studies were carried out in hypertensive overweight and obese subjects with type 2 diabetes, characteristics representative of patients treated with GLP-1R agonists in a clinical setting. Furthermore, we were able to assess changes in BP in the same group of subjects, enabling an assessment of whether changes in ANP or urinary sodium were associated with potential reduction of BP. Our data clearly demonstrate that liraglutide increased urinary sodium excretion independent of changes in circulating ANP or reduction of SBP. These findings, taken together with the lack of significant SBP reduction over 3 weeks in the same patient population, suggest that enhanced urinary sodium excretion alone may be insufficient

to explain the BP-lowering effects described with liraglutide and other GLP-1R agonists (7,13).

Intriguingly, an observational non-randomized study of 31 obese (BMI 31.7 kg/m^2), prehypertensive (mean BP 138.2/85.9 mmHg) subjects with type 2 diabetes reported significant increases in plasma levels of ANP and Nt-proBNP after 12 weeks of daily liraglutide administration (14). Furthermore, the magnitude of changes in ANP and Nt-proBNP correlated with the extent of weight loss after 12 weeks. In a secondary analysis, we observed robust changes in plasma ANP concentrations in four patients after acute administration of liraglutide. Taken together, these findings are consistent with the known heterogeneity in control of ANP secretion and the pathophysiology of hypertension in human subjects (15) and leave open the possibility that a small subset of hypertensive subjects may exhibit increased ANP secretion after administration of GLP-1R agonists. Furthermore previous studies have demonstrated that levels of circulating natriuretic peptides may be increased after weight loss secondary to lifestyle changes (16,17) or bariatric surgery (18). Hence, although the available data suggest that acute GLP-1R activation does not stimulate secretion of ANP or Nt-proBNP in humans, chronic therapy with GLP-1R agonists may indirectly increase circulating levels of ANP and Nt-proBNP in obese subjects through incompletely understood mechanisms related to weight loss.

Our study has several limitations. At study entry, the majority of patients were receiving concomitant therapy with single or multiple antihypertensive agents, including 55% receiving adjuvant diuretic therapy. Hence, one or more of these antihypertensive agents may have impacted the effect of liraglutide to modulate ANP secretion, urinary sodium excretion, or BP. Second, we did not measure dietary changes in sodium intake. As patients lost body weight following 21-day liraglutide treatment, sodium intake may have increased or decreased with liraglutide treatment. As several study end points, such as plasma ANP levels and urinary sodium excretion, are sensitive to changes in dietary sodium intake, this represents a major potential limitation in study

Table 1—Within-subject treatment difference for liraglutide compared with crossover treatment with placebo on 24-h ABPM

Variable	Baseline median (ICR)	Treatment group liraglutide (1.8 mg) 21 days, (n = 17) LSMD from baseline (±SE)	Treatment group placebo 21 days, (n = 17) LSMD from baseline (±SE)	Treatment difference liraglutide (1.8 mg) vs. placebo LSMD (±SE), P value
SBP (mmHg)				
24 h	130.45 (136.19, 127.10)	+1.62 ± 2.04	−0.72 ± 2.04	+2.33 ± 1.67, P = 0.18
Daytime	133.07 (141.70)	+1.68 ± 2.14	−0.58 ± 2.14	+2.25 ± 1.78, P = 0.23
Nighttime	122.73 (129.69, 118.58)	+0.87 ± 2.64	−0.83 ± 2.64	+1.70 ± 2.77, P = 0.54
DBP (mmHg)				
24 h	79.74 (83.05, 68.79)	+3.39 ± 1.12	−0.39 ± 1.12	**+3.78 ± 1.34, P = 0.01**
Daytime	79.67 (85.44, 70.48)	+3.66 ± 1.24	+0.19 ± 1.24	+3.48 ± 1.48, P = 0.03
Nighttime	71 (75.75, 63.98)	+2.32 ± 1.46	−1.44 ± 1.46	**+3.77 ± 1.74, P = 0.05**
HR (bpm)				
24 h	67.59 (86.17, 63)	+7.39 ± 2.08	+2.18 ± 2.08	**+5.21 ± 2.42, P = 0.05**
Daytime	73.32 (89.68, 64.93)	+6.51 ± 2.28	+1.94 ± 2.27	+4.57 ± 2.69, P = 0.11
Nighttime	63.25 (76.02, 58.53)	+9.73 ± 1.77	+2.39 ± 1.77	**+7.34 ± 2.38, P = 0.008**
MAP (mmHg)				
24 h	97.03 (100.32, 91.26)	+1.71 ± 1.83	−0.60 ± 1.83	+2.31 ± 2.18, P = 0.30
Daytime	101.43 (103.09, 93.74)	+2.65 ± 1.48	−0.06 ± 1.48	+2.71 ± 1.53, P = 0.10
Nighttime	89.57 (95.07, 84.56)	−0.10 ± 1.53	+1.21 ± 1.54	−1.32 ± 2.18, P = 0.55
PP (mmHg)				
24 h	54.62 (61.76, 46.88)	−1.99 ± 1.36	−0.76 ± 1.36	−1.22 ± 1.33, P = 0.37
Daytime	54.75 (63.07, 47.42)	−0.98 ± 0.94	−1.77 ± 0.94	+0.78 ± 1.33, P = 0.56
Nighttime	52.64 (60.12, 46.13)	−1.45 ± 1.63	+0.62 ± 1.63	−2.07 ± 1.50, P = 1.19

Baseline data are presented as median (ICR), and the baseline-subtracted within-subject difference for 21-day liraglutide treatment compared with 21-day treatment with placebo are presented as LSMD ± SE. Boldface indicates statistical significance. ICR, interquartile range; LSMD, least squares mean difference; MAP, mean arterial pressure; PP, pulse pressure.

design. Furthermore, our study population was predominantly overweight or obese middle-aged males with established diabetes and hypertension, and it remains unclear whether other cohorts of diabetic subjects may have responded differently to liraglutide.

Three principal features of our study include the small sample size, brief study period, and the achievement of maximally approved liraglutide dosing (1.8 mg) for only the last week of the study. These limitations may partially explain why we were unable to detect significant changes in plasma ANP levels or SBP in this patient population. Nevertheless, our BP findings are in agreement with the results of a trial examining the BP-lowering effects of twice-daily exenatide, which reported trends toward lowering of SBP, daytime DBP, and nighttime BP; however, none of the differences reported were statistically significant (19). In contrast, Ferdinand et al. (20) randomized 755 patients to dulaglutide or placebo and reported a significant reduction in SBP after 16 weeks in 251 subjects randomized to receive 1.5 mg of dulaglutide administered once weekly. Furthermore, several large outcome studies have demonstrated significant reductions in SBP

following 26-week or longer treatment with liraglutide against placebo and active comparator controls (21–23). Hence, the small sample size of the current exploratory study or the short exposure to maximal treatment with liraglutide (1.8 mg for only 7 days) may have limited detection of small changes in SBP associated with liraglutide.

We demonstrate that overweight or obese subjects with type 2 diabetes and hypertension exhibit significant increases in urinary sodium excretion in response to sustained liraglutide administration, independent of concomitant changes in BP or circulating levels of natriuretic peptides. Hence, the antihypertensive mechanism(s) engaged by GLP-1R signaling in this patient population are independent of a cardiorenal GLP-1–ANP axis described in rodents (12). Our findings provide new insight into temporal pharmacodynamic changes arising in hypertensive diabetic subjects treated with liraglutide and refine our understanding of the relationships between GLP-1R–dependent increases in urinary sodium and potential reductions in BP. In contrast, the chronotropic actions of liraglutide were easily detected in our study; however, the increment in HR

was transient and was no longer statistically significant 4 h after the last dose of liraglutide. Collectively, our data emphasize that the mechanisms linking activation of GLP-1R signaling to control of BP in hypertensive diabetic humans remain incompletely understood and require further investigation.
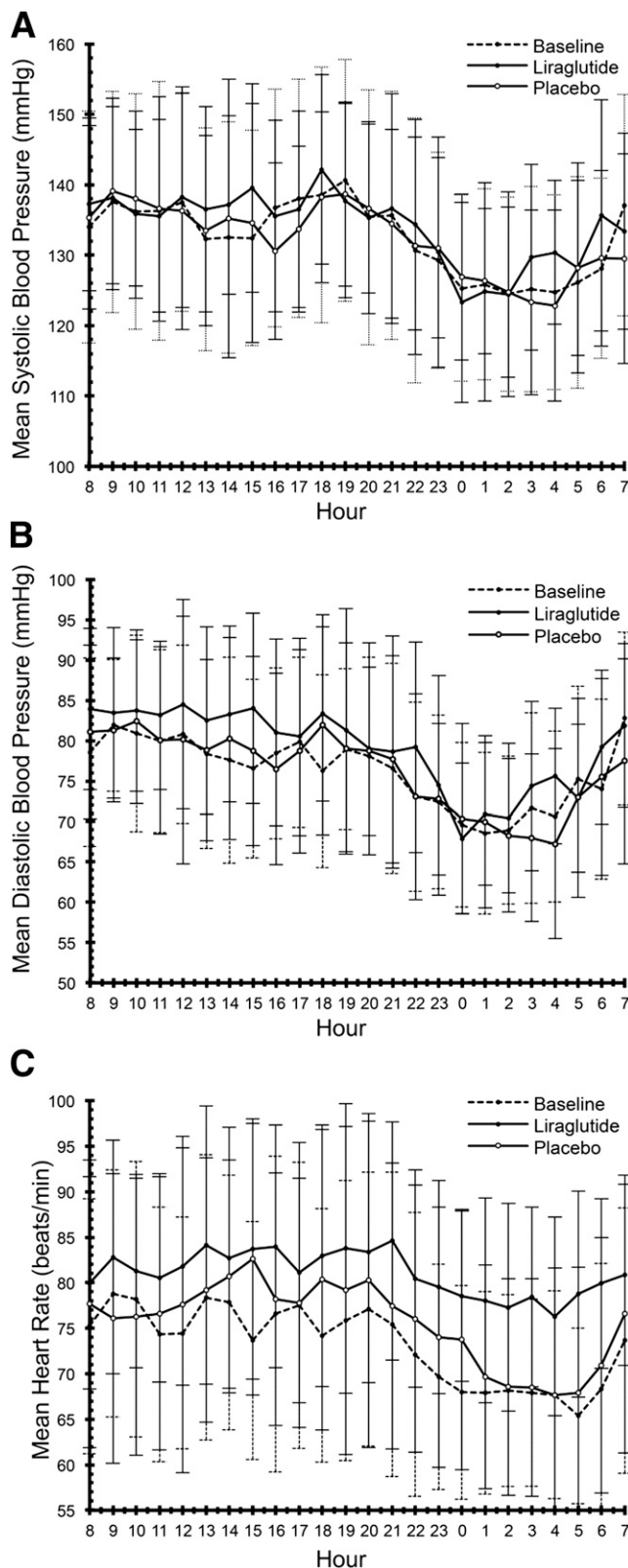
Figure 3—Effect(s) of 21-day liraglutide treatment on mean 24-h ambulatory BP and HR compared with crossover treatment with placebo. Twenty four–hour ABPM was used to measure hourly SBP (*A*), DBP (*B*), and HR (*C*) during a 24-h period at baseline and following 21-day treatment with liraglutide and with placebo. Data are presented as hourly means (±SD). Solid black line with square markers represents liraglutide (1.8 mg), solid gray line with triangular markers represents placebo, and dashed line with triangular markers represents baseline. Least-squares mean difference in 24-h ABPM is presented in Table 1.

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SUPPLEMENTARY DATA

Supplementary Table 1. Baseline Patient Characteristics

Variable	Sub-Variable	Baseline (n=18)
Gender (male/female)		14/4
Average Age (yrs)		62.3 (65.0, 56.4)
Ethnicity (%)	Caucasian (%)	56
	Black (%)	17
	South Asian (%)	11
	Indian (%)	17
Body Weight (Kg)		91.65 (110.5, 76.03)
Body Mass Index (Kg/m^2)		29.50 (35.14, 26.78)
Office Systolic BP (mmHg)		144
Office Diastolic BP (mmHg)		85
Office Heart Rate (bpm)		73
24-Hr Systolic BP (mmHg)		130.28 (136.39, 125.07)
24-Hr Diastolic BP (mmHg)		79.74 (83.05, 68.79)
24-Hr Heart Rate (bpm)		73.78 (12.08)
HbA$_{1c}$ % (mmol/mol)		7.0 (53)
Serum Creatinine (µmol/L)		79
eGFR (ml/min/1.73m^2)		82.64
24-Hr Urine Microalbumin (mg/24hrs)		15.45 (61.43, 9.23)
24-Hr Urine ACR (mg/mmol)		1.38 (3.14, 0.84)
24-Hr Urine Protein (mg/24hrs)		121.50 (174.00, 91.5)
24-Hr Urine PCR (mg/mmol)		9.09 (13.10, 6.83)
Medical History	Diabetes Duration (yrs)	5.8
	Hypertension Duration (yrs)	9.7
	Prior MI (%)	10
OHA Use	metformin use (%)	94
	sulphonylurea use (%)	33
Anti-Hypertensives Use	beta blocker (%)	30
	diuretic (%)	55
	calcium antagonists (%)	60
	ACE inhibitor (%)	75
	ARB (%)	20
	alpha blocker (%)	5
#/Anti-Hypertensives	0 (drug naïve) (%)	44
	1 (%)	17
	2 (%)	17
	3 (%)	11
	4 (%)	0
	5 or more (%)	11
Nocturnal Dipping Status	Dipper (%)	29
	Non-Dipper (%)	53
	Riser (%)	18
Other Medication Use	Statin (%)	61
	Anti-Platelet (%)	55
	Proton Pump Inhibitors (%)	17

Normally distributed data are presented as mean (±SD), data that were not of a normal distribution are presented as median (ICR). The baseline characteristics were assessed at the screening visit, and 24-Hr urine and blood pressure data during the Baseline Test Period, n=18 (except for 24-Hr blood pressure measurements where n=17)).

Abbreviations: ACE, angiotensin converting enzyme; **ARBs**, angiotensin II receptor blockers; **bpm,** beats/min; **cm,** centimeters; **eGFR**, estimated glomerular filtration rate; **ICR**, interquartile range; **Kg**, kilograms; **m**, meters; **mg**, milligrams; **mmHg**, millimeters of mercury; **MI**, myocardial infarction; **mL**, millilitres; **mmol**, millimoles; **OHA**, oral hypoglycemic agent; **µmol,** micromoles; **yrs**, years.

SUPPLEMENTARY DATA

Supplementary Table 2. The effect of 21 day liraglutide treatment on metabolic parameters and serum biochemistry compared to cross-over treatment with placebo

	Treatment Group		Treatment Group		liraglutide
	liraglutide (n=18)		placebo (n=18)		vs. placebo
	Clinic Visits		Clinic Visits		
Variable	1 day	21 days	1 day	21 days	LSMD (±SE)
HbA1c (%)	7.04 ± 0.091	6.61 ± 0.087	6.9 ± 0.076	7.06 ± 0.089	-0.7 ± 0.0, p=0.005
Fasting Glucose (mmol/L)	8.39 ± 2.94	5.68 ± 1.42	8.43 ± 3.04	8.54 ± 2.60	-3.4 ± 0.74, p=0.0004
Total Cholesterol (mmol/L)	4.16 ± 1.03	3.64 ± 0.94	3.98 ± 1.00	4.1 ± 1.02	-0.63 ± 0.17, p=0.002
LDL Cholesterol (mmol/L)	2.00 ± 0.92	1.78 ± 0.84	1.9 ± 0.86	2.07 ± 0.99	-0.37 ± 0.16, p=0.04
Triglycerides (mmol/L)	1.62 ± 1.19	1.43 ± 0.80	1.51 ± 0.75	1.52 ± 0.73	-0.2 ± 0.18, p=0.28
HDL (mmol/L)	1.20 ± 0.40	1.11 ± 0.32	1.18 ± 0.41	1.18 ± 0.39	-0.083 ± 0.03, p=0.02
CRP (mg/L)	2.48 ± 2.83	2.15 ± 2.33	3.29 ± 4.63	2.6 ± 2.77	0.09 ± 0.80, p=0.91
Sodium (mmol/L)	139.67 ± 1.81	139.78 ± 1.83	139.44 ± 2.38	139.33 ± 2.25	0.20 ± 0.80, p=0.80
Potassium (mmol/L)	4.12 ± 0.42	4.07 ± 0.44	4.21 ± 0.42	4.19 ± 0.41	-0.30 ± 0.14, p=0.84
Chloride (mmol/L)	102.28 ± 3.16	101.39 ± 2.68	101.67 ± 2.95	101.72 ± 2.63	-0.89 ± 0.77, p=0.27
Urea (mmol/L)	6.32 ± 1.60	5.88 ± 1.25	6.62 ± 1.96	6.39 ± 1.73	-0.21 ± 0.32, p=0.53
Creatinine (mmol/L)	80.94 ± 18.17	84 ± 19.71	82.94 ± 20.48	82.06 ± 22.05	+4.11 ± 2.19, p=0.08
eGFR (ml/min/1.73m2)	83.22 ± 16.93	79.44 ± 15.76	82.22 ± 16.42	83.72 ± 17.45	**-5.76 ± 2.60, p=0.04**
ANG II (pmol/L)	2.47 ± 1.19	2.93 ± 2.39	2.71 ± 1.94	3.73 ± 4.10	-0.97 ± 0.86, p=0.28
Cortisol (mmol/L)	402.78 ± 97.61	376.11 ± 120.50	409.44 ± 113.27	425.56 ± 178.30	-41.75 ± 49.31, p=0.41
TSH (mIU/L)	2.34 ± 1.83	2.28 ± 2.15	2.38 ± 1.68	2.37 ± 1.76	-0.05 ± 0.15, p=0.77

Treatment group means are presented as mean ± SD, and the within-subject difference (change from Day 21-Day1) compared to cross-over treatment with placebo is presented as the LSMD ± SE.

Abbreviations: ANG II, angiotensin II; **CRP**, c-reactive protein, eGFR, estimated glomerular filtration rate**HbA1c**, glycosylated haemoglobin A1c; **HDL**, high-density lipoprotein, **IU**, international units; **LDL**, low-density lipoprotein, and **LSMD**, least squares mean difference; mg/L, milligrams per litre; **mmol/L**, millimoles per litre; **SE**, standard error, **SD**, standard deviation; **TSH**, thyroid stimulating hormone.

SUPPLEMENTARY DATA

Supplementary Table 3. The effect of 21 day liraglutide treatment on anthropometric and hemodynamic parameters measured in the office at clinic visits compared to cross-over treatment with placebo

Variables	Treatment Group liraglutide (n=18) Clinic Visits		Treatment Group placebo (n=18) Clinic Visits		liraglutide vs. placebo
	Day 1	21 Day	Day 1	21 Day	LSMD (±SE), p-value
Anthropometric Measurements					
Body Weight (Kg)	97.01 ± 31.1	95.99 ± 31.60	96.46 ± 31.33	96.79 ± 31.32	**-1.35 ± 0.46, p=0.009**
Waist Cir. (cm)	105.86 ± 18.90	106.30 ± 20.51	106.66 ± 20.02	105.82 ± 20.78	+1.31 ± 0.92, p=0.17
BMI (Kg/cm²)	32.01 ± 8.08	31.64 ± 8.20	32.00 ± 8.26	32.05 ± 8.22	**-0.42 ± 0.18, p=0.03**
Height (cm)	172.88 ± 12.84	172.81 ± 12.95	172.31 ± 12.57	172.52 ± 12.78	-0.17 ± 0.24, p=0.5
Office Hemodynamic Measurements					
SBP (mm Hg), Time = 0 hrs	137.83 ± 15.09	133.33 ± 11.16	134.22 ± 9.17	132.39 ± 10.23	-2.35 ± 3.49, p=0.51
SBP (mm Hg), Time = 4 hrs	142.2 ± 12.45	139.05 ± 14.30	137.83 ± 10.34	135.59 ± 13.90	-0.34 ± 3.77, p=0.93
DBP (mm Hg), Time = 0 hrs	83.11 ± 7.45	85.38 ± 7.26	83.55 ± 9.13	82.28 ± 9.72	+3.6 ± 2.33, p=0.14
DBP (mm Hg), Time = 4 hrs	85.85 ± 9.24	87.55 ± 8.16	85.3 ± 9.72	85.84 ± 10.99	+1.64 ± 1.80, p= 0.38
MAP (mm Hg), Time = 0 hrs	101.35 ± 8.30	101.37 ± 6.66	100 ± 7.32	98.94 ± 8.82	+1.62 ± 2.61, p=0.54
MAP (mm Hg), Time = 4 hrs	103.85 ± 8.13	103.69 ± 6.68	101.98 ± 8.17	101.31 ± 9.19	+0.98 ± 2.10, p=0.66
HR (bpm), Time = 0 hrs	70.33 ± 11.84	78.88 ± 11.37	71.28 ± 9.60	70.28 ± 13.36	**+9.25 ± 3.51, p=0.02**
HR (bpm), Time = 4 hrs	67.06 ± 9.78	73.66 ± 9.95	65.72 ± 9.32	68.94 ± 14.90	+3.69 ± 3.02, p=0.24

Treatment group means are presented as mean ± SD, and the within-subject difference (change from Day 21-Day1) compared to cross-over treatment with placebo is presented as the LSMD ± SE.

Abbreviations: BMI, body mass index; **bpm**, beats per minute; **cm**, centimeters; **DBP**, diastolic blood pressure; **HR**, heart rate; **Hr**, hour, **Kg**, kilograms; **MAP**, mean arterial pressure; **mmHg**, millimeters of mercury; **SBP**, systolic blood pressure, **SD**, standard deviation; SE, standard error.

SUPPLEMENTARY DATA

Supplementary Table 4. Adverse Events

	Placebo (n=20)	Liraglutide (n=20)
Any AE	3 (15%)	13 (65%)
Any AE leading to inability to titrate	0	1 (5%)
AE reported:		
Loss of Appetite	0	6 (30%)
Nausea	0	4 (20%)
GI Bloating	0	2 (10%)
Vomiting	0	1 (5%)
Loss of Energy	0	2 (10%)
Flatulence	0	3 (15%)
Abdominal Pain	1 (5%)	0
Dyspepsia	0	3 (15%)
Constipation	0	1 (5%)
Diarrhea	2 (10%)	0
Tachycardia	0	0
Exertional tachycardia	1 (5%)	0
Cervical Lymph Node	1	1 (5%)

Data presented as absolute numbers and percentages are provided in brackets. Included are each type of AE reported during treatment with either placebo group or with liraglutide for all 20 subjects who participated in the research study.

Abbreviations: AE, Adverse Event; **GI**, gastrointestinal.

SUPPLEMENTARY DATA

## Supplementary Figure 1A. Study Design and Timeline
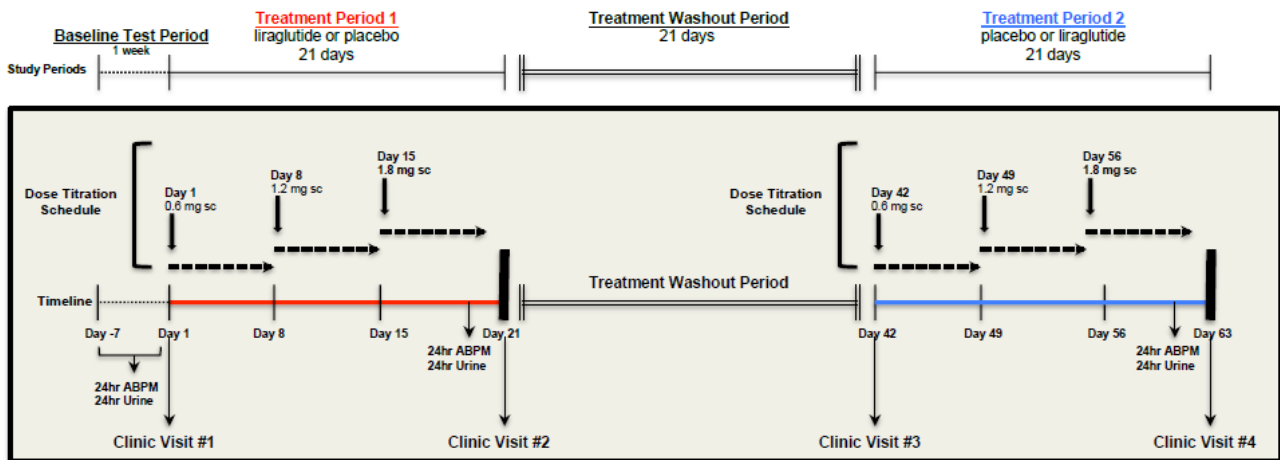
1A) Patients entered the study during the Baseline Test Period, which occurred -7 to -1 days prior to Treatment Period 1. During the Baseline Test Period, patients completed a 24-Hr ABPM and a 24-Hr urine collection. Patients then entered Treatment Period 1 on **Day 1** and presented to clinic for Clinic Visit #1. At Clinic Visit #1, office BP and HR was measured (Time = 0hrs) by an automated oscillometric device, and a venous canula was placed to allow access for blood sampling. Patients received their first dose of study drug at a dose of 0.6 mg sc which they continued daily (denoted by the dashed lines). On **Day 8**, the daily dose of study drug was increased, and was titrated weekly (**Day 8 and Day 15**) to a final dose of 1.8 mg as indicated. After study drug administration, blood was sampled hourly for 4 hours **(2b).** After 21 days of treatment, patients returned for Clinic Visit #2 (all clinic visits identical), and then entered the 21 day Treatment Washout Period. On **Day 42**, patients entered Treatment Period 2, where they crossed over to receive reciprocal treatment to Treatment Period 1. Patients received their first dose of study drug at Clinic Visit #3 and continued daily administration and weekly dose titration (occurring on **Day 49, and Day 56**) for 21 days until Clinic Visit#4 which marked the end of Treatment Period 2 and the end of the study.

Abbreviations: ABPM, ambulatory blood pressure monitoring; **ANP**, atrial natriuretic peptide; **BP**, blood pressure; **hrs**, hours; **Hr**, hour, **HR**, heart rate, **mg**, milligrams; **sc**, sub-cutaneous.

SUPPLEMENTARY DATA

**Supplementary Figure 1B. Experimental Study Protocol**
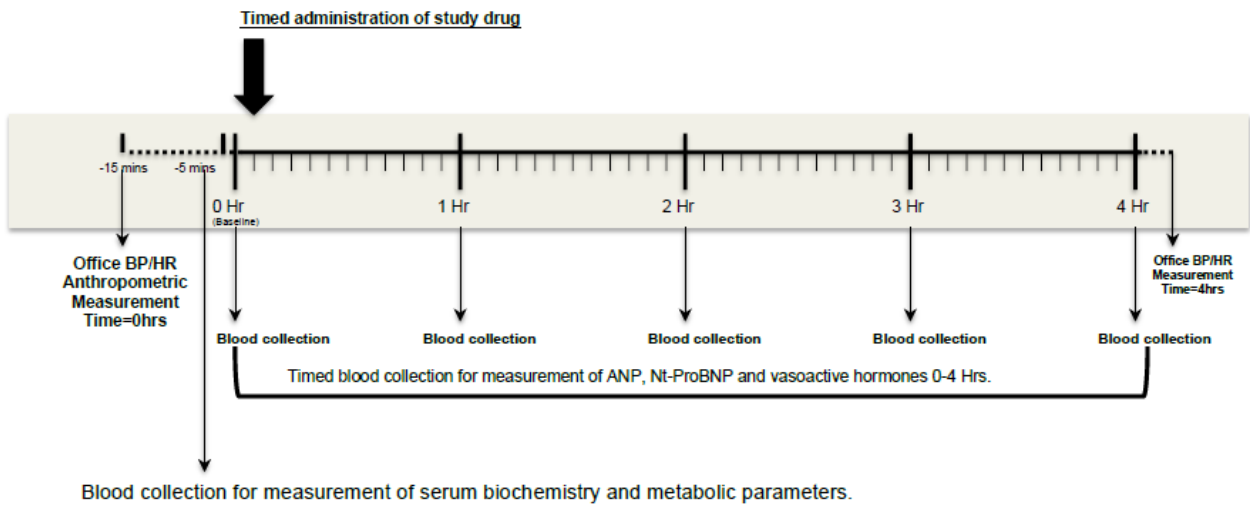
1B) At all Clinic Visits, patients underwent blood sampling before (-5 minutes, 0) and hourly (1, 2, 3, 4 hours) after administration of study drug for measurement of baseline and dynamic changes in plasma ANP/Nt-proBNP, serum biochemistry and vasoactive hormone concentrations in response to study drug administration.

Abbreviations: ANP, atrial natriuretic peptide; **BP**, blood pressure; **HR**, heart rate; **Nt-proBNP**, N-terminal pro-B-type natriuretic peptide.

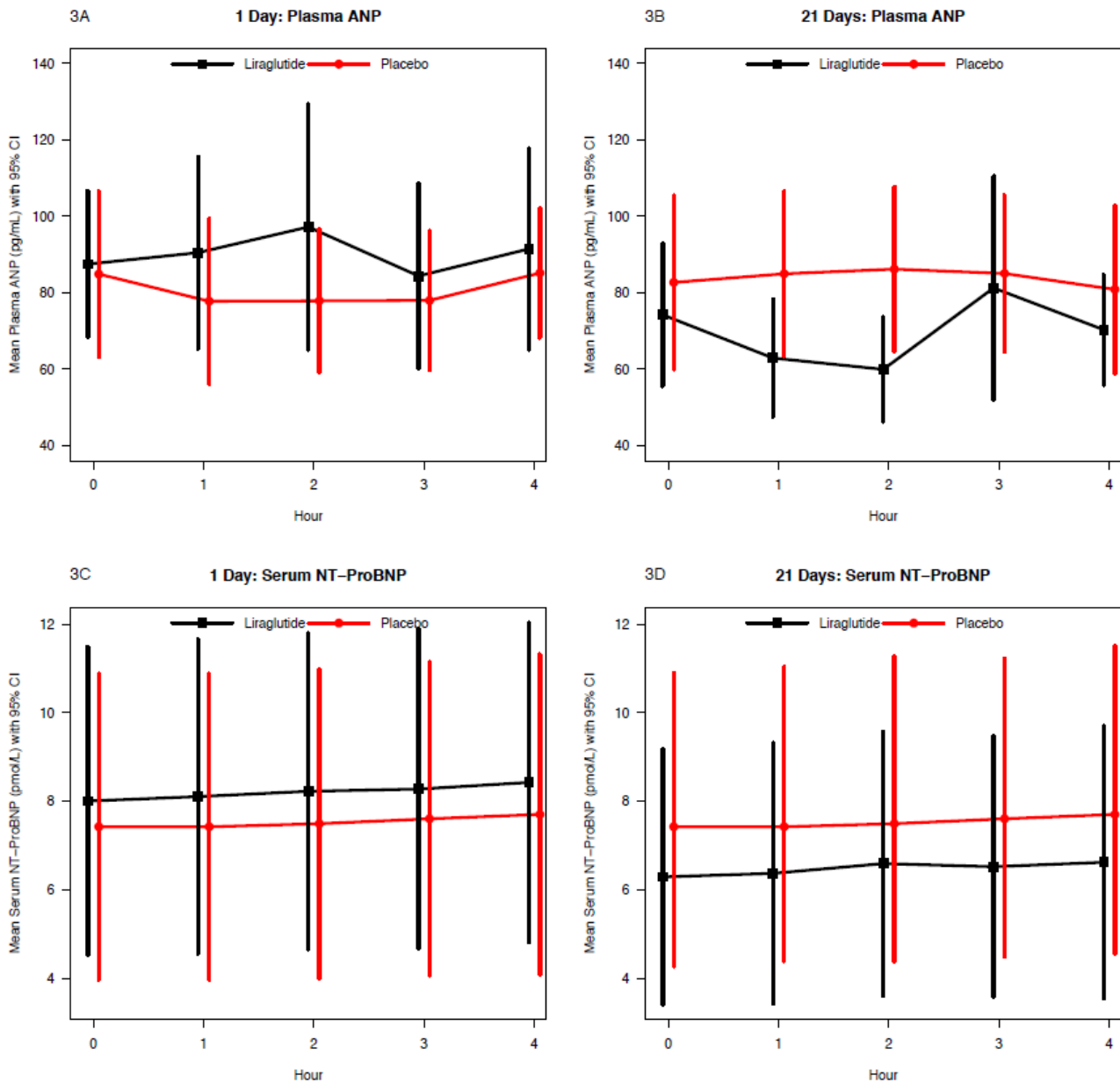SUPPLEMENTARY DATA

Supplementary Figure 2. Patient Flow Diagram
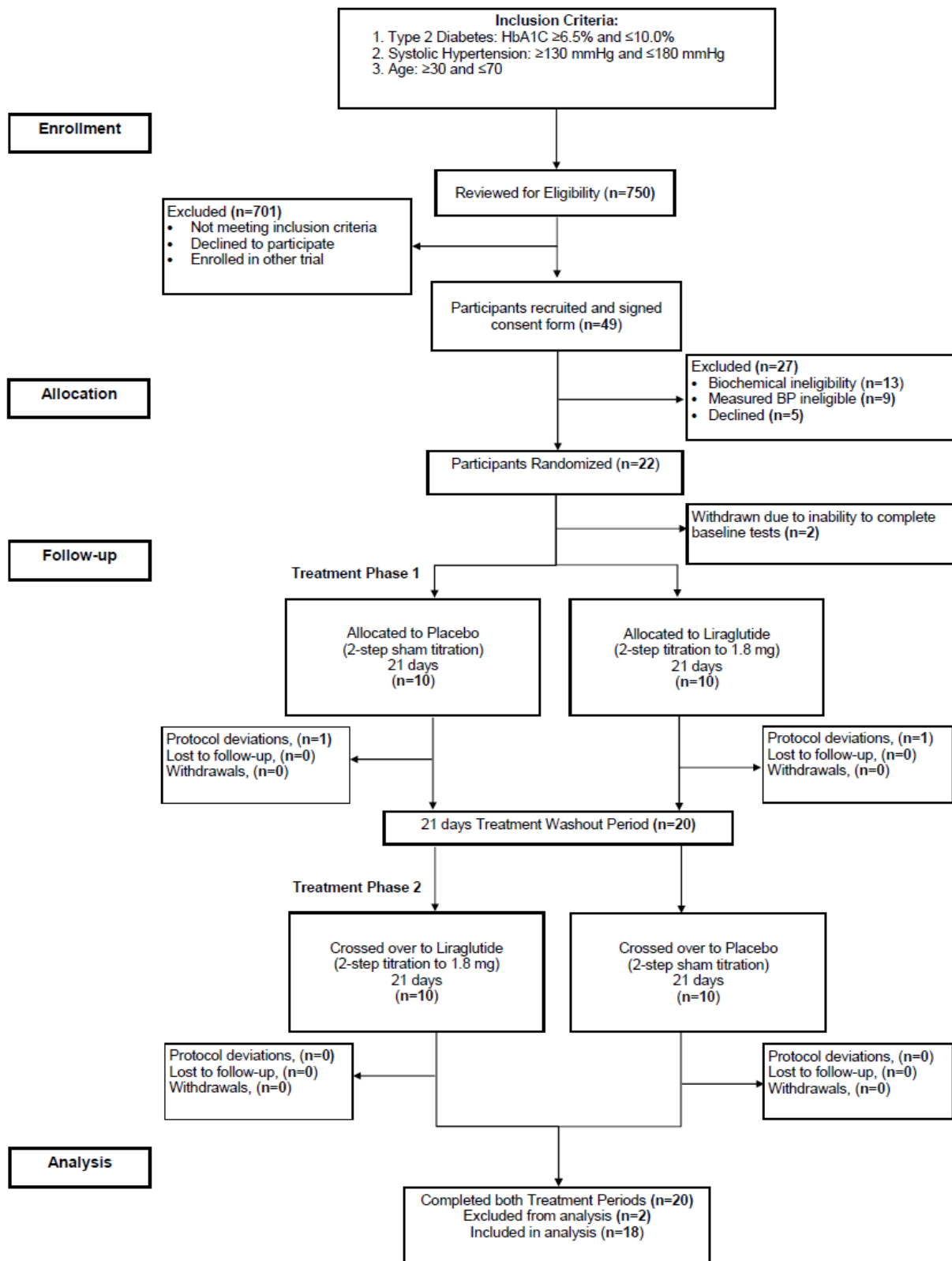
Supplementary Figure 3. Effect of 1 day or 21 day treatment with liraglutide on cardiac natriuretic peptide concentrations compared to cross-over treatment with placebo.


Blood was collected from subjects and plasma ANP levels were measured hourly following 1 day (**3A**) and following 21 days (**3B**) of treatment with liraglutide and with placebo. Presented are the mean plasma ANP (pg/mL) concentrations with 95% CI. Mean serum Nt-ProBNP concentrations were also measured following 1 day (**3C**) and following 21 days (**3D**) of treatment with liraglutide and with placebo. Presented are the mean serum Nt-ProBNP (pmol/L) concentrations with 95% CI.

Treatment Doses: 1 day (0.6 mg), 21 day (1.8 mg).

Abbreviations: ANP, atrial natriuretic peptide; **BNP**, b-type natriuretic peptide; **CI**, confidence interval; **Nt-ProBNP**, N-terminal proBNP.

Supplementary Figure 4. The effect of 21 day liraglutide treatment on 24-Hr, daytime, and nighttime urinary albumin-tocreatinine ratio compared to cross-over treatment with placebo
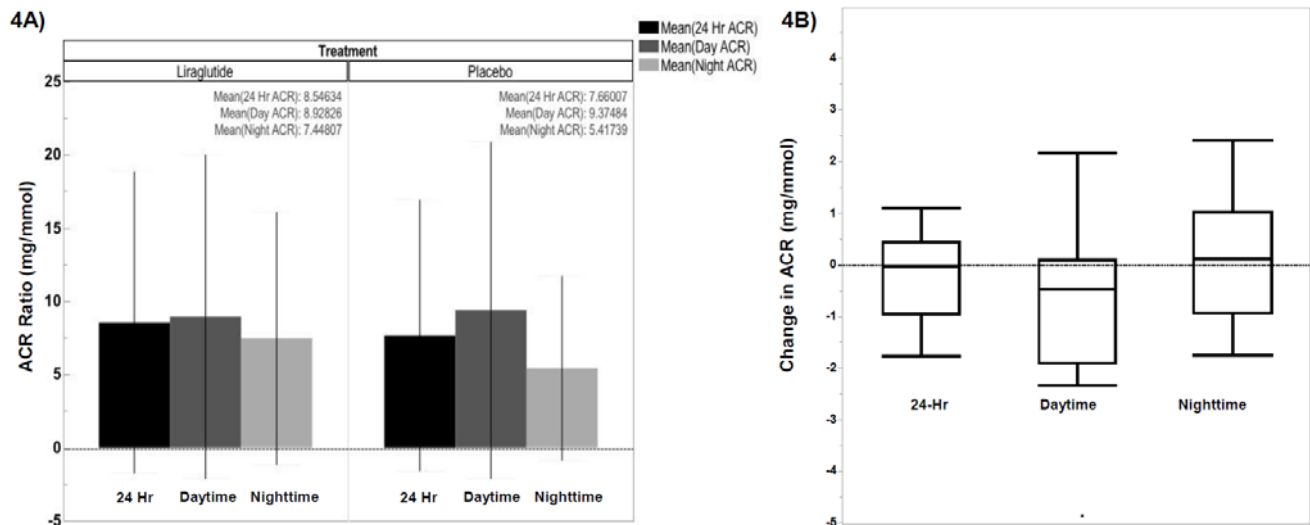
The effect of 21 day liraglutide treatment on 24-Hr, daytime, and nighttime urinary sodium excretion compared to cross-over treatment with placebo

The mean (95% CI) 24-Hr, daytime, nighttime urinary albumin-to-creatinine ratio for the treatment groups (liraglutide, placebo) is presented in **4A**. The within-subject change (LSMD) in the 24-Hr, daytime, nighttime urinary albumin-to-creatinine ratio from baseline for liraglutide compared to placebo (from baseline) are presented in **4B**.

For the Box plots and whiskers graph, horizontal line indicates the median change, and the box represents interquartile range for the change, and outliers are presented as single points.

Nonparametric tests were used for comparison (Wilcoxon rank-sum); *, denotes the normal approximation for 2-sided p-value <0.05; ** denotes the normal approximation for 2-sided p-value <0.005 for liraglutide compared to placebo (baseline-subtracted).

Abbreviations: ACR, albumin-to-creatinine ratio; **CI**, confidence interval, **Hr**, hour, **LSMD**, Least squares mean difference; **mg**, milligrams; **mmol**, millimoles.