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Lixisenatide versus insulin glulisine on fasting and postbreakfast systemic haemodynamics in type 2 diabetes mellitus patients

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Abstract

The prolonged treatment effects of a short-acting glucagon-like peptide-1 receptor agonist (GLP-1RA), such as lixisenatide, on fasting and postprandial systemic haemodynamics in type 2 diabetes mellitus patients are unknown. In this secondary analysis, we included 34 overweight insulin glargine–treated type 2 diabetes mellitus patients (mean±SD age, 62±7 years; HbA_{1c}, 8.0±0.9%; systolic blood pressure [BP], 133.9±16.1 mm Hg; diastolic BP, 75.4±8.39 mm Hg) that were randomised to once-daily lixisenatide 20 μg or once-daily titrated insulin glulisine for 8 weeks. Systemic haemodynamics (oscillometric device and finger photoplethysmography), arterial stiffness (applanation tonometry), and cardiac sympathovagal balance (heart rate variability) were measured in the fasting state and repetitively (up to minute 175) after a standardised mixed breakfast. Acetaminophen was given orally to estimate gastric emptying rate. Lixisenatide did not affect fasting systemic haemodynamics compared with insulin glulisine from baseline to week 8. Postbreakfast overall, lixisenatide compared with insulin glulisine tended to increase systolic BP by 5.2±2.9 mm Hg (p=0.087) and increased diastolic BP by 5.4±1.4 mm Hg (p<0.001), with respective maximal differences of +10.2±3.7 mm Hg (p=0.007) and +7.2±1.5 mm Hg (p<0.001). Lixisenatide increased systemic vascular resistance (*p*<0.001) and arterial stiffness (p=0.007). No between-group differences in overall postbreakfast heart rate, cardiac output, or cardiac sympathovagal balance, and circulating catecholamines, angiotensin II, or aldosterone were observed. Both treatments lowered HbA_{1c} similarly, whereas lixisenatide achieved greater reductions in postbreakfast plasma glucose excursions. Lixisenatide slowed gastric emptying rate, which statistically explained changes in postbreakfast BP. Lixisenatide compared with once-daily titrated insulin glulisine for 8 weeks does not affect fasting but increases postbreakfast BP in insulin glargine–treated type 2 diabetes mellitus patients. This effect could, at least in part, be explained by reduced passage rate of nutrients and water and activation of the gastrovascular reflex.

Introduction

Glucagon-like peptide-1 (GLP-1) receptor agonists (RA's) mimic the effects of the native gut-derived incretin hormone GLP-1, and are now routinely used as glucose-lowering drugs in type 2 diabetes mellitus (T2DM) management.[1] GLP-1RA's can be categorized as either short-acting compounds, which provide short-lived receptor-activation, or as long-acting compounds, which continuously activate GLP-1 receptors (GLP-1R).[1] The differences in pharmacokinetic profiles translate into different clinical actions. For instance, short-acting GLP-1RA's predominantly lower postprandial glucose excursions by slowing gastric emptying rate (GER) and reducing intestinal glucose uptake and glucagon release, whereas long-acting GLP-1RA's have stronger effects on fasting glucose levels, mediated predominantly by their insulinotropic actions.[1]

Consistent with the wide distribution of the GLP-1R, GLP-1RA's are known to exhibit multiple extrapancreatic effects, including actions on the heart and blood vessels.[2] Immediate administration of GLP-1 or a GLP-1RA increases fasting blood pressure (BP) in many human studies.[3-9] In contrast, prolonged GLP-1RA treatment in T2DM patients is generally associated with lowering of fasting BP[10] by ≈1.8 to 4.6 mmHg.[11] Underlying mechanisms remain unclear, yet may involve bodyweight-lowering, natriuresis/diuresis, endothelial-dependent/independent vasodilation, decreased arterial stiffness, and/or reduced renin-angiotensin-aldosterone-system (RAAS)-activity.[10,12]

Although humans spent most of diurnal time in the postprandial state, the effects of GLP-1RA-treatment on BP after meal ingestion remains largely unstudied. In T2DM patients, immediate infusion of GLP-1[13] or GLP-1RA exenatide[14] attenuated the BP lowering response to orally administered glucose or a mixed-meal, respectively. Several mechanisms are proposed to explain this effects. As such, the GLP-1RA-induced reduction in GER may prevent a postprandial BP fall by slowing the passage rate of nutrients to the small intestine,[13,14] and by increasing vascular resistance in muscles through gastric distension (the so-called gastro-vascular reflex).[13-17] Moreover, increased cardiac output (CO),[18] sympathetic nervous system (SNS)-activity[19] and vascular stiffness,[20] and reduced insulin-mediated vasodilation[1,14,16] may play part. Postprandial BP augmentation is of interest as observational studies suggest that postprandial hypertension is associated with atherosclerosis.[21] However, it is unclear whether the increase in postprandial BP with GLP-1RA's is sustained over time. We demonstrated that the long-acting GLP-1RA liraglutide does not affect repeatedly measured postprandial BP and systemic haemodynamic functions in T2DM patients after 12 weeks of treatment.[14] In contrast to liraglutide, we recently showed that 8-week treatment with lixisenatide, a prandial short-acting GLP-1RA, increases postbreakfast BP at a single postmeal time-point in overweight T2DM patients compared with once-daily titrated insulin glulisine (iGlu).[12] In the current secondary analysis of this trial, we aimed to further detail the effects of lixisenatide on fasting and postprandial BP responses, and explore potential mechanisms that drive changes in BP. We hypothesised that prolonged lixisenatide-treatment compared with iGlu increases postbreakfast BP by reducing GER and circulating glucose and insulin concentrations, leading to raised systemic vascular resistance (SVR) and arterial stiffness, and by increasing CO.

Methods

Trial design

This was a prespecified secondary analysis of a phase-4, monocentre, randomised, open-label, comparator-controlled, parallel-group, intervention trial, which was primarily designed to determine the effects of lixisenatide versus once-daily titrated iGlu on renal physiology; design and detailed methods have been published previously.[12] After a run-in period of 4 weeks, during which antihyperglycaemic treatment was not changed, subjects were randomised and entered an intervention period of 8 weeks. After week 4, a safety visit was performed. The study protocol, protocol amendments and any other protocol-specific documents were approved by the ethics review board of the VU University Medical Centre (Amsterdam, The Netherlands) and local authorities. The study complied with the Declaration of Helsinki and Good Clinical Practice guidelines, and was registered at URL: http://www.clinicaltrials.gov. Unique identifier: NCT02276196). The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study population

Eligible patients were white men or postmenopausal women, aged 35 to 75 years with T2DM and a body mass index >25 kg/m^2. Patients were treated with basal insulin glargine, with or without metformin. BP needed to be controlled (i.e. <140/90 mmHg) and treatment for hypertension and/or albuminuria included a renin-angiotensin-system (RAS)-inhibition. Exclusion criteria included a recent (<6 months) history of cardiovascular disease (including immediate coronary syndrome, heart failure or stroke), complaints compatible with gastroparesis or the use of diuretics that could not be stopped 3 months before and during the intervention period.

Details of the randomisation process and interventions are contained in the Supplemental methods.

Endpoint measurements

Patients were instructed to control their intake of sodium (9–12 g/day) 2 days before to testing procedures, to abstain from vigorous physical activity and alcohol ingestion for ≥24 hours, and to withhold from nicotine or caffeine for ≥12 hours. Patients arrived in the fasting state and delayed all morning medications with the exception of metformin and thyroid hormone replacement therapy. A venous cannula was inserted in an antecubital vein of the non-dominant arm for venous blood sampling. All measurements were performed after a quiet rest period of at least 10 minutes in supine position, and at the non-dominant arm, which was placed comfortably at heart level. Appropriate cuff sizes were used were applicable.

Measurements were performed ≈85 to 50 min before, and repeated up to 175 minutes after a standardised mixed breakfast, which consisted of bread with cheese and jam, and milk (416 kcal; 14 g fat; 48 g carbohydrates; and 22 g protein). Together with the meal, 1.5 g of acetaminophen in suspension form was administered to determine GER.[12] After the fasting measurements at week 8, patients injected lixisenatide or iGlu 30 and 10 minutes before breakfast, respectively.

To stimulate diuresis for the renal tests that were performed concurrently,[12] patients consumed 500 mL of tap water before arriving at the clinical research unit and 10 mL/kg (maximum 1000 mL) of tap water in 2 equal parts just prior and ≈40 minutes after the meal, followed by 150 mL ≈70 and ≈115 minutes postprandially. Fasting blood samples were analysed for HbA_{1c}, plasma glucose, hematocrit, creatinine and renin-angiotensin-aldosterone-system (RAAS) hormones (i.e plasma renin concentration [PRC], angiotensin-II, and aldosterone). RAAS hormones and hematocrit were also determined 105 and 115 minutes after the meal, respectively. Catecholamines (i.e. noradrenaline, adrenaline and dopamine) were determined 105 minutes postprandially, whereas blood glucose, acetaminophen (as indicated in Figure 5), insulin and C-peptide levels were measured throughout the testing day.[12]

After randomisation, patients were instructed to consume a daily breakfast with a comparable composition as offered during the testing day in order to titrate iGlu.

Blood pressure and heart rate

Systolic BP (SBP), diastolic BP (DBP)) and heart rate (HR) were measured by a trained observer using an automatic oscillometric device (Dinamap®, GE Healthcare, Little Chalfont, United Kingdom). Measurements were performed in triplicate at 1 to 2-minute intervals and the mean of the last 2 measurements was used for each time point (as indicated in Figure 1 and Figure 3).[22] If the second and third measurement were discrepant, a fourth measurement was taken.[22] The overall postbreakfast SBP and DBP were the endpoints of primary interest. For safety, patients returned to the CRU at week 4, during which fasting BP and HR were also measured

Systemic haemodynamic functions

Stroke volume (SV), cardiac output (CO) and systemic vascular resistance (SVR) were calculated non-invasively by a beat-to-beat finger arterial photoplethysmography BP-monitoring device (Nexfin®, Amsterdam, The Netherlands). SV, CO and SVR were normalised for body surface area, to acquire SV index (SVI), cardiac index (CI) and SVR index (SVRI), respectively. The finger BP-measurements were performed over a period of 30 seconds, and a mean was derived using dedicated software (Nexfin@PC version 2.0, BM Eye, Amsterdam, the Netherlands). The within-day and between-day variability of these assessments are ≤9.1%.[8]

Pulse wave analysis

Pulse wave analysis (PWA) was performed at the level of the radial artery using applanation tonometry with a high-fidelity micromanometer (SPT-301, Millar Instruments, Houston, TX, USA) coupled to a SphygmoCor® apparatus and version 6.31 software (Atcor Medical Pty Ltd, West Ryde, Australia). The average of two measurements of ≥12 seconds was used, which needed to have adequate pulse wave profiles and a high quality control, defined as an in-device quality index of >80%. The central aortic pressure waveform was derived from the radial artery waveform using the software's mathematical transfer function.[23,24] The augmentation index (an

indicator of arterial stiffness) was calculated as the augmentation pressure, that is the pressure of the second systolic peak minus the pressure at the inflection point, expressed as percentage of the pulse pressure and normalised for a HR of 75 bpm (AIX@HR75).

Heart rate variability

Using an ECG-equipped Nexfin© device, 5-minute RR-interval recordings were obtained, during which patients were instructed to breath spontaneously (range 10-18 breaths/min) and to refrain from sleeping or speaking. ECG-measurements were visually inspected and artifacts were manually corrected using linear interpolation. ECG-recordings were loaded into Kubios heart rate variability (HRV) analysis software 2.2 (University of Eastern Finland, Biosignal Analysis and Medical Imaging Group, Kuopio, Finland). After additional automated low level artifact correction and removal of trend components (smoothn priors setting), Fast Fourier spectral analyses were performed to obtain normalised low frequency (LF, 0.04 to 0.15 Hz) and high frequency (HF, 0.15 to 0.5 Hz) bands, from which the LF/HF-ratio, a validated marker for cardiac sympathovagal-balance,[9] was calculated.

Autonomic nerve function testing, body impedance analysis (for assessment of body fat and body water) and laboratory analyses are described in the Supplemental methods.

Calculations, data management and statistical analyses

As current data are part of a larger study, of which the mechanistic effects on systemic haemodynamics were added as secondary endpoints, no formal sample size calculation was performed for this objective. The absolute area under the curve (AUC) after the meal was calculated for glucose, insulin and acetaminophen, and the maximal acetaminophen concentration (C_{max}) and the time to reach C_{max} (T_{max}) were noted. Finger arterial BP-measurements were discarded in one patient randomised to lixisenatide because of failure of the sensor. Postprandial hypotension was defined as a fall in SBP of >20 mmHg within 2 hours after the meal.[16] Statistical analyses were performed in the per protocol using SPSS Statistics for Windows, V22.0 (IBM Corp, Armonk, NY). For single-measured parameters, multivariable linear regression models were used to examine lixisenatide-induced effects compared with iGlu. To correct for potential between-group baseline differences, corresponding baseline-values were added as independent variable. Within-group comparisons were analysed using paired t-tests or Wilcoxon signed rank tests, as appropriate. Linear mixed model analyses were used to analyse both the between- and the within-group changes of repeatedly-measured parameters. To assess the overall postbreakfast between-group treatment effect, treatment allocation and the averaged corresponding baseline values (to adjust for baseline group differences) were added to the model. To assess overall postbreakfast within-group changes, treatment allocation was added as a categorical variable with the corresponding baseline values as reference category. To assess between- and within-group treatment effects at specific fasting and postbreakfast time-points, time (treated as a categorical variable represented by dummy variables) and the interaction between treatment allocation and time were added to the model.

Additional analyses were performed in which the influence of predefined parameters that could theoretically be of influence (AUC of acetaminophen, glucose and insulin, and the LF/HF-ratio, AIX@75 and CI) and differed between- or within-groups was assessed on postbreakfast SBP and DBP by adding the week 0 to week 8 difference as covariate. In addition, the interaction with RAS-inhibitors was explored by adding RAS-inhibitor use and the interaction between treatment allocation and RAS inhibitor use to the model. Spearman signed rank test was used to assess associations. Data are presented as mean±SEM, unless stated otherwise. Statistical significance was considered at a 2-sided p-value <0.05.

Results

Patient characteristics

The patient selection process has been described.[12] After exclusion of 1 patient in each treatment-group because of pre-existent atrial fibrillation, the final study population for the current analysis comprised of 18 patients in the iGlu-arm and 16 patients in the lixisenatide-arm (Supplemental Figure 1). Demographic and baseline clinical characteristics are presented in the Table. The median [IQR] iGlu-dose at week 8 was 17 [8-29] units/day.

Fasting measurements

In the fasting state and before injection of the treatment drugs, lixisenatide compared with iGlu did not affect SBP (+1.5±3.7 mmHg; p=0.682), DBP (+0.3±1.7 mmHg; p=0.864), HR, SVI, CI, SVRI, AIX@75 or the LF/HF-ratio from baseline to week 8 (p>0.1) (Figure 1 and Figure 2). These parameters also did not change within-groups, except for an SBP reduction in the iGlu arm (-6.9±2.8 mmHg; p=0.015) (Supplemental Figure 2). After 4 weeks, there were no between or within-group differences in fasting SBP, DBP or HR (p>0.05; data not shown).

Postbreakfast measurements

Blood pressure and heart rate

At baseline in all patients, meal ingestion did not affect SBP, whereas DBP decreased at all but the last time point with a maximum difference of -2.5±0.8 mmHg (p=0.002) at minute-55. Postprandial hypotension occurred in three patients, all subsequently randomised to iGlu. From baseline to week 8, lixisenatide compared with iGlu tended to increase overall SBP by +5.2±2.9 mmHg (p=0.087) with a maximal difference of +10.2±3.7 mmHg (p=0.007) (Figure 1 and Figure 3). Within-group, lixisenatide increased SBP with an overall difference of 6.4±1.4 mmHg (p<0.001) and maximally 9.1±2.9 (p=0.002) (Supplemental Figure 2). At week 8, none of the patients had postprandial hypotension. From baseline to week 8, overall DBP increased with lixisenatide compared with iGlu by 5.4±1.4 mmHg (p<0.001) with a maximal difference of +9.5±1.8 mmHg (p<0.001). Within-group, lixisenatide increased overall DBP by 5.5±0.7 mmHg (p<0.001) and maximally by 7.2±1.5 mmHg (p<0.001). Lixisenatide compared with iGlu did not alter overall HR (+0.5±1.5 bmp; p=0.748), but tended to increase HR at minute-175 (+3.0±1.8

Table. Baseline characteristics

Variables	Insulin glulisine (N=18)	Lixisenatide (N=16)
Age, years	61±7	62±7
Male, *n* (%)	11 (61)	11 (69)
Current smoker, *n* (%)	4 (22)	3 (19)
Bodyweight, kg	93.9 [79.1-108.9]	96.4 [88.3-110.1]
BMI, kg/m^2	31.0 [27.9-34.9]	30.6 [27.5-35.6]
Waist circumference, cm^2	115.5 [107.0-122.1]	109.5 [105.5-118.1]
Diabetes mellitus duration, years	14±8	11±5
Insulin use duration, years	5 [2-11]	4 [1-8]
Insulin glargine dose, units	39 [32-51]	44 [42-54]
HbA_{1c}, %	7.8±0.8	8.4±0.8*
HbA_{1c}, mmol/mol	62±9	68±9*
Fasting plasma glucose, mmol/L	7.3±2.1	6.7±1.9
eGFR (CKD-EPI 2009), mL/min/1.73 m^2	82±11	90±10*
Systolic blood pressure, mmHg	138.1±17.7	129.7±13.1
Diastolic blood pressure, mmHg	76.8±9.9	73.7±7.5
Heart rate, bpm	68±10	64±9
Autonomic dysfunction, *n* (%)	4 (24)	8 (50)
Metformin use, *n* (%)	16 (89)	15 (94)
Statin use, *n* (%)	11 (61)	9 (56)
Antihypertensive medication use, n (%)	11 (61)	10 (63)
Beta blocker use, n (%)	6 (33)	5 (31)
Calcium channel blocker use, n (%)	5 (28)	5 (31)
Alfa blocker use, n (%)	2 (11)	1 (6)
RAS inhibitor use, n (%)	11 (61)	10 (63)
ACE inhibitor use, n (%)	6 (33)	8 (50)
ARB use, n (%)	5 (28)	2 (13)

Data are mean±SD, median [IQR], or count (percentage). *P<0.05 for between-group difference using appropriate tests. ACE, angiotensin-converting enzyme; ARB, angiotensin-II receptor blocker; BMI, body mass index; CKD-EPI, chronic kidney disease epidemiology collaboration; eGFR, estimated glomerular filtration rate; RAS, renin-angiotensin-system.

bpm; p=0.096). Within-group, lixisenatide increased overall HR by 1.2±0.6 bpm (p=0.029) and by 3.3±1.1 bpm at minute-175 (p=0.003).

Systemic haemodynamic functions

From baseline to week 8, lixisenatide compared with iGlu decreased overall SVI by 2.2±0.8 mL/1.73 m^2 (p=0.010) with a maximal difference of -4.3±1.1 mL/1.73 m^2 (p<0.001) (Figure 1 and Figure 3). Lixisenatide also decreased SVI within-group (Supplemental Figure 2). Lixisenatide compared with iGlu did not change overall CI (p=0.120), although CI decreased at minute-90 by 0.25±0.09 L/min/1.73 m^2 (p=0.010). CI did not change within the treatment groups. Overall SVRI increased with lixisenatide compared with iGlu by 316±60 dyn·sec/cm/1.73 m^2 (p<0.001) with a maximal difference of +435±84 dyn·sec/cm/1.73 m^2 (p<0.001). Within-group, overall SVRI increased with lixisenatide (p<0.001) and decreased with iGlu (p=0.039).

Pulse wave analysis

After 8 weeks, lixisenatide compared with iGlu increased overall AIX@75 by 3.1±1.1 (p=0.007) (maximally +4.3±1.3; p=0.001) (Figure 2 and Figure 4). Within-group, overall AIX@75 was higher with lixisenatide (p=0.005) and reduced with iGlu (p=0.026; Supplemental Figure 2).

Heart rate variability

There were no between or within-group differences in the LF/HF-ratio overall (p=0.999) or at single time-points after 8 weeks (Figure 2, Figure 4 and Supplemental Figure 2).

Metabolic and hormonal measurements

Lixisenatide and iGlu reduced HbA_{1c} from baseline to week 8 by 0.8±0.1% (p<0.001) and by 0.6±0.1%(p=0.001) respectively; there was no between-group difference (p>0.05). Fasting glucose did not change after 8 weeks between or within treatment-arms (p>0.05; Supplemental Table 1). Compared with iGlu, lixisenatide reduced the glucose AUC (p=0.001; Figure 5). There were few measurements in the hypoglycaemic range (Figure 5). Lixisenatide compared with iGlu reduced the acetaminophen AUC (p<0.001) and C_{max} (p<0.001), whereas increasing T_{max} (p=0.010; Figure 5). Insulin (endogenous and insulin glargine) reduced within-group with lixisenatide (p=0.003) and did not change with iGlu or between-groups. C-peptide decreased in both treatment-arms with no between-group differences (Supplemental Table 1). There were no within or between-group differences in circulating postbreakfast angiotensin-II, aldosterone or catecholamine levels.

Bodyweight and body composition

Numerically, bodyweight reduced with lixisenatide and increased with iGlu from baseline to week 8; the between-group difference of 1.4±0.6 kg reached statistical significance (p=0.022; Supplemental Table 1). There were no differences in fasting or postbreakfast body water percentage. There was no between-group difference in fasting hematocrit, while postbreakfast hematocrit slightly increased with lixisenatide compared with iGlu (+0.01±0.01; p=0.045; Supplemental Table 1).

Exploratory analyses

In linear mixed models, the overall postbreakfast change in SBP was –to a large extent– explained by differences in acetaminophen AUC (mean difference reduced from 5.2±2.9 mmHg to 2.9±3.8 mmHg; significance level changed from p=0.087 to p=0.453), and –to a minor extent– explained by differences in AIX@75 (to 4.5±3.0 mmHg; p=0.141), but not by glucose AUC (to 6.3±32 mmHg; p=0.060) or insulin AUC (to 5.0±3.2; p=0.120). The overall postbreakfast difference in DBP was largely explained by differences in acetaminophen AUC (from +5.4±1.4 mmHg to +3.9±1.8 mmHg; p<0.001 to p=0.042), but not by AIX@75 (to 5.2±1.4 mmHg; p=0.001), glucose AUC (to +5.6±1.5 mmHg; p=0.001) or insulin AUC (to 5.2±1.5; p=0.001). There was no interaction between the overall postbreakfast effect of SBP or DBP with RAS-inhibitor use (p=0.806 and p=0.739, respectively).

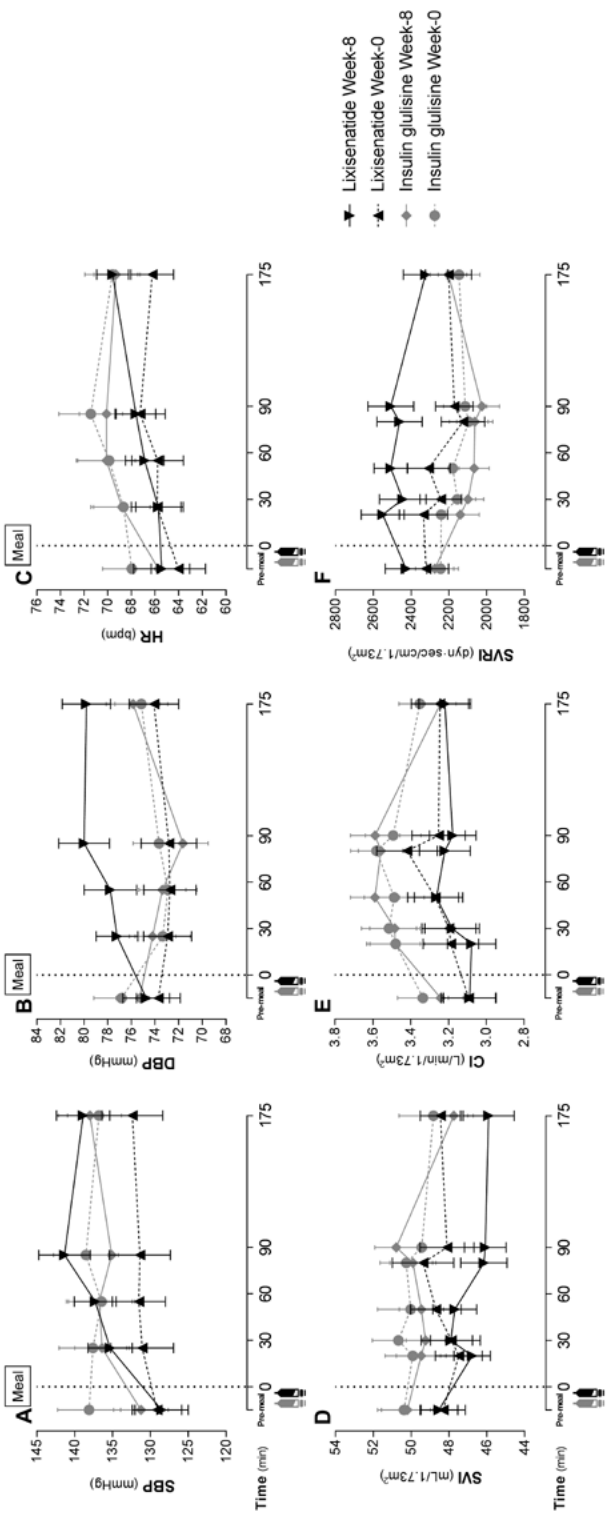


Figure 1. Blood pressure, heart rate and systemic haemodynamics functions measurements at baseline and after 8-week treatment with lixisenatide or insulin glulisine. Data are mean±SEM. CI, cardiac index; DBP, diastolic blood pressure; HR, heart rate; SBP, systolic blood pressure; SVI, stroke volume index; SVRI, systemic vascular resistance index.

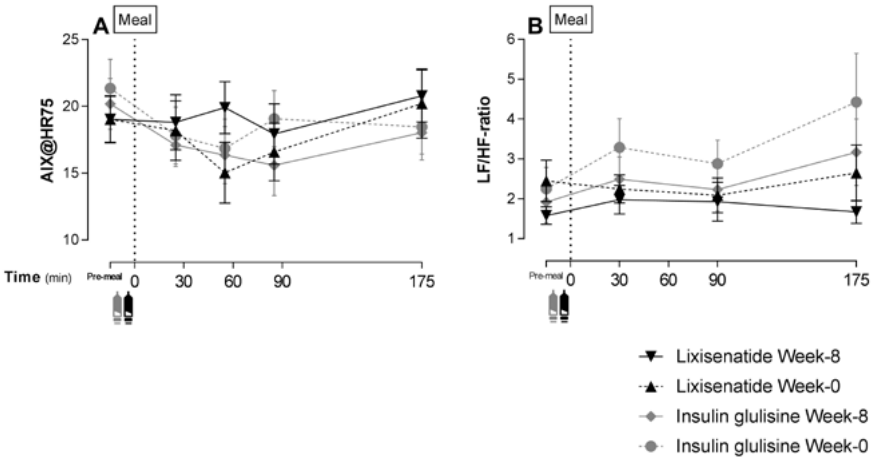


Figure 2. Pulse wave analysis and heart rate variability measurements at baseline and after 8-week treatment with lixisenatide or insulin glulisine. Data are mean±SEM. AIX@HR75, augmentation index normalised to heart rate of 75 bpm; LF/HF-ratio, ratio of low-frequency to high frequency.

In correlation analyses in all patients, the maximal changes in SBP and DBP at minute-85 associated with differences in acetaminophen AUC (r: -0.42; p=0.017 and r: -0.61; p<0.001, respectively) and acetaminophen levels at minute-90 (r: -0.38; p=0.034 and r: -0.58; p<0.001; Supplemental Figure 3).

Discussion

In this secondary analysis of a randomised controlled trial, we demonstrated that prolonged treatment with lixisenatide compared with once-daily titrated iGlu does not affect fasting, but increases overall postbreakfast BP, SVRI and arterial stiffness in overweight T2DM patients on insulin glargine. The lixisenatide-induced vasopressor response was paralleled by a decrease in SVI, without changes in overall postbreakfast CI or cardiac sympathovagal-balance (HRV), or circulating catecholamines, angiotensin-II or aldosterone.

Lixisenatide did not affect fasting BP, despite inducing significant reductions in bodyweight and a sustained natriuretic effect after breakfast.[12] The neutral effect on fasting BP in the current study is in contrast with the reported 0.8 mmHg SBP-reduction in the 25-month landmark cardiovascular outcome trial (CVOT) of lixisenatide (ELIXA; Evaluation of Lixisenatide in Acute Coronary Syndrome), involving 6,068 patients with T2DM and a recent acute coronary event.[25] The differential outcomes likely reflect the use of an active comparator (i.e. iGlu lowered SBP within-group in our study), used sample size and/or duration of follow-up.

The lixisenatide-induced vasopressor response after breakfast is in line with previous studies, in which immediate GLP-1- or exenatide-infusion attenuated the hypotensive response after oral ingestion of a meal or glucose in older volunteers[13] and T2DM patients.[13,14] Notably, 8 week lixisenatide treatment did not affect mean 24-h measured SBP or DBP in T2DM patients,[26]

suggesting that the drug increases postprandial BP only during a single meal, without meaningful effects on 24-h BP. In contrast, in our recent study in T2DM patients, 12-week treatment with the long-acting GLP-1RA did not affect postprandial systemic haemodynamics.[14] The neutral effect of liraglutide could be because of GLP-1R tachyphylaxis or drug-induced activation of compensatory pathways with long-acting GLP-1RA's, which does not occur with short-acting GLP-1RA's. This results in waning effects on GER[26] (also resulting in higher postprandial glucose and insulin) and potentially likewise on other homeostatic systems that influence postprandial BP (see below). Alternatively, the differential response between lixisenatide and liraglutide on BP might involve structural diversities, as lixisenatide shares ~50% amino acid sequence identity with mammalian GLP-1, whereas this is 97% for liraglutide.[12]

The long-term clinical significance of a postbreakfast increase in BP with lixisenatide, and potentially also with other short-acting GLP-1RA's, is unknown. Observational studies suggest that arteriosclerosis is higher in elderly subjects that show a postprandial hypertensive response (defined as an SBP-increase of >10 mmHg within 30 minutes after a standardised lunch).[21] Moreover, although the clinical relevance of a "transient" postprandial AIX response is uncertain, a higher AIX in the fasting state is associated with increased cardiovascular and renal risk.[27,28] Nevertheless, the ELIXA trial established the cardiovascular safety or neutrality of the drug,[25] implying that the changes do not importantly impact clinical outcome, potentially because of their transient nature. Alternatively, although T2DM patients in ELIXA were relatively frail compared with other CVOT's, thus leaving little room for cardiovascular improvement, we can speculate that the observed vasopressor response partly explains the neutral effect on cardiovascular events with a short-acting GLP-1RA[25] compared with the cardiovascular benefits seen with the long-acting GLP-1RA's liraglutide[29] and semaglutide[30] and to lesser extent exenatide once-weekly.[31]

Yet, in a specific T2DM population, a transient meal-related increase in BP could be clinically beneficial in the prevention of postprandial hypotension. Postprandial hypotension occurs in 0% to 70% of T2DM patients[32] and is associated with syncope, falls, angina, dizziness, light-headedness, visual disturbance and increased mortality.[16] Interestingly, a proof-of-concept study in 15 healthy older subjects and 15 T2DM patients showed that acute administration of lixisenatide 10 μg markedly reduced GER of a 75 g glucose load and consequent glycaemia, which was associated with attenuation of a rise in splanchnic blood flow and fall in postprandial SBP.[33] Notably, although we aimed for real-life effects in this study, the additional water ingestion as part of the protocol presumably led to higher postbreakfast BP estimates (see below), leading to potential overestimation of the corresponding cardiovascular and renal risk, and limiting adequate assessment of postprandial hypotension. Moreover, although the absence of morning anti-hypertensive medication permitted characterization of the true effects of the study drugs, results may be different when BP medication is present.

Numerous mechanisms may explain the observed increase in postbreakfast BP. As such, the lixisenatide-induced reduction in GER may have induced a direct vasopressor response. After a meal, the interaction of nutrients with the small intestine triggers a potential BP-fall by splanchnic blood pooling and consequent reduction in venous return of blood to the heart,

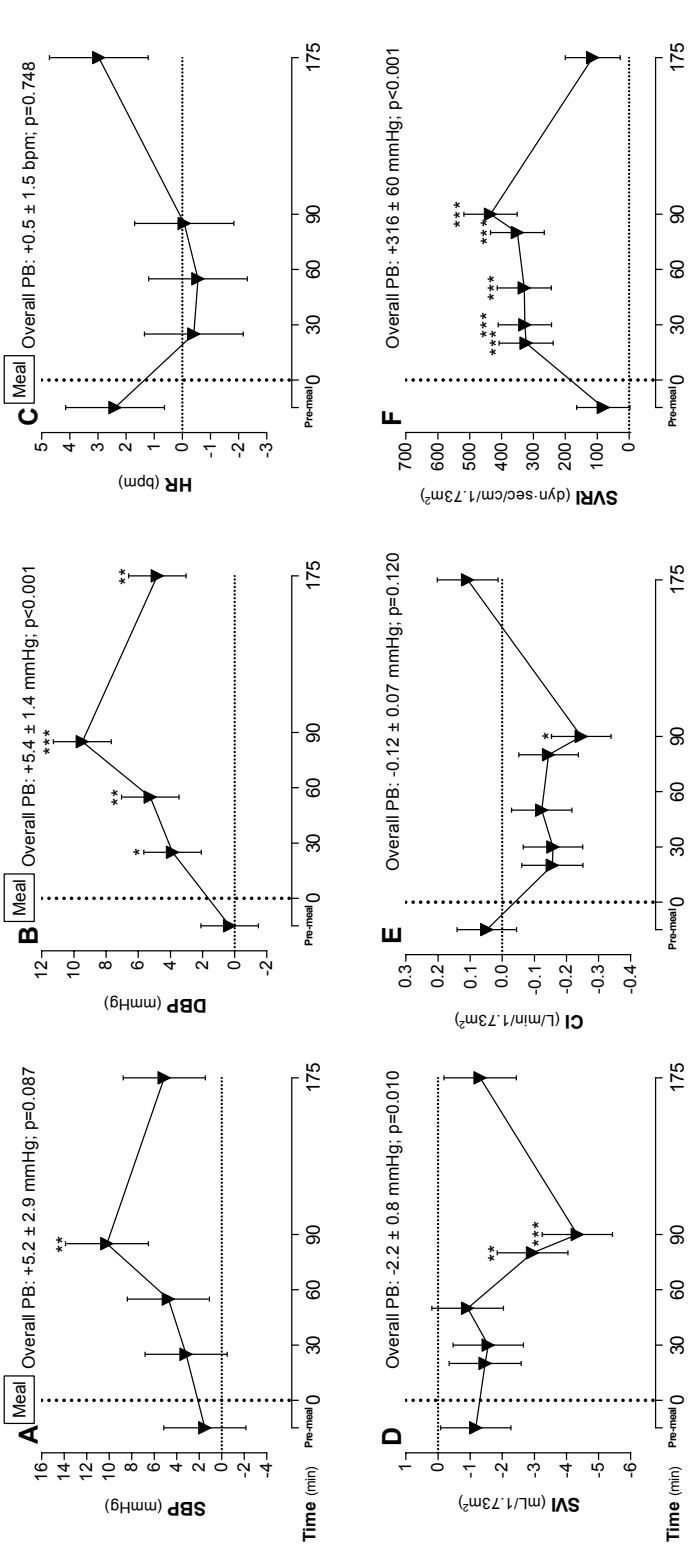


Figure 3. Mean differences in blood pressure, heart rate and systemic haemodynamics functions of 8-week treatment with lixisenatide compared with insulin glulisine. Data are mean difference±SEM using linear mixed models to examine baseline-corrected lixisenatide-induced effects compared with insulin glulisine. *p<0.05, **p<0.01, and ***p<0.001. CI, cardiac index; DBP, diastolic blood pressure; HR, heart rate; PB, postbreakfast. SBP, systolic blood pressure; SVI, stroke volume index; SVRI, systemic vascular resistance index.

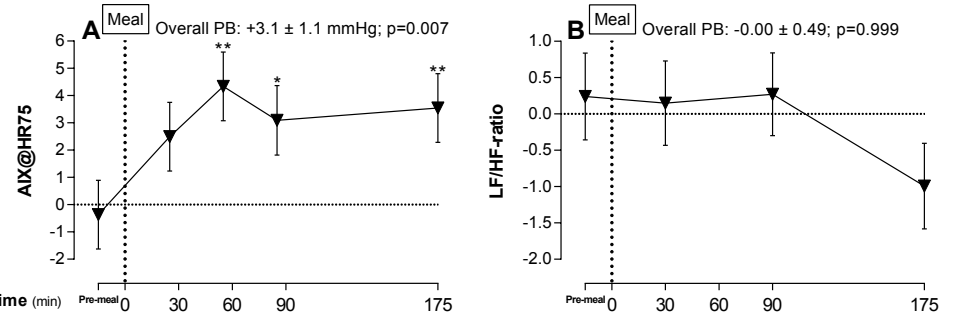
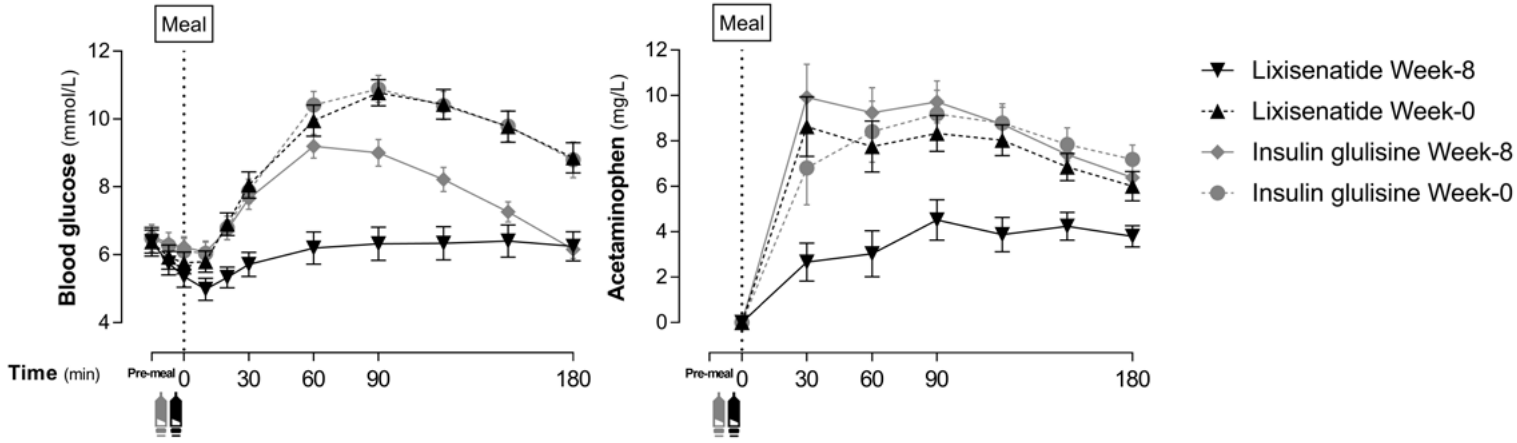


Figure 4. Mean differences in pulse wave analysis and heart rate variability of 8-week treatment with lixisenatide compared with insulin glulisine. Data are mean difference±SEM using linear mixed models to examine baseline-corrected lixisenatide-induced effects compared with insulin glulisine. *p<0.05 and **p<0.01. AIX@HR75, augmentation index normalised to heart rate of 75 bpm; LF/HF-ratio, ratio of low-frequency to high frequency; PB, postbreakfast.

presumably mediated by neural and humeral factors.[16,34] This response is greater when GER is relatively more rapid in T2DM.[35] However, postprandial BP-lowering is attenuated by gastric distension, which increases muscle sympathetic nervous activity (MSNA), vascular resistance and consequently BP (the so-called gastro-vascular reflex).[15-17] Interestingly, ingestion of 480 mL water to induce gastric distension markedly increases SBP in patients with severe autonomic failure[36,37] and older controls,[37] whereas it attenuates the hypotensive response to a meal in patients with postprandial hypotension.[38] Notably, HR decreased in these studies.[36-38] The effect is not explained by changes in intravascular volume or hormones, as it occurs within minutes after water ingestion and is almost completely abolished by ganglion blockage, indicating a neuronal pathway.[37] Adding guar gum to a meal also slows GER and small intestinal nutrient absorption leading to an attenuated postprandial BP-reduction in older subjects and T2DM paitents.[16] In the current study, we hypothesise that lixisenatide not only prevented a fall in postbreakfast BP by inhibiting the passage rate of nutrients through the stomach and small bowel, but also augmented the gastro-vascular reflex through nutrient- and water-induced gastric distension. Notably, the fast-acting neural pathways that may directly increase muscle sympathetic nerve activity do not necessarily associate with markers for cardiac and systemic SNS-activity, that is the LF/HF-ratio and circulating catecholamines, respectively,[17] which did not change in the current study. However, in studies that investigated the haemodynamic effects of intraduodenal glucose infusion, thereby bypassing the stomach, exogenous GLP-1 also attenuated the hypotensive response in healthy older subjects,[39] whereas exenatide even increased BP in T2DM patients.[18]

These findings indicate that the effects of lixisenatide are, at least in-part, mediated through mechanisms other than the gastro-vascular reflex. First, it has been suggested that the GLP-1-related increase in HR raises CI and thereby BP.[3,7,8,18,40] Alternatively, GLP-1R-mediated inhibition of parasympathetic outflow or augmentation of SNS-activity could increase CI.[3,5,19,41] Notably, preclinical studies also suggest that GLP-1R-stimulation increases



Blood glucose area under the curve (mmol/L/3h)

	Insulin glulisine	Lixisenatide
Week-0	1693±66	1678±65
Week-8	1415±54**	1096±72**
Mean difference 'Lixisenatide-insulin glulisine	-	-318±90**

Acetaminophen area under the curve (mg/L/3h)

	Insulin glulisine	Lixisenatide
Week-0	1288 [677-1762]	1131 [970-1723]
Week-8	1527 [1011-1778]	500 [244-830]**
Mean difference Lixisenatide-insulin glulisine	-	-804±138***

Figure 5. Blood glucose and acetaminophen responses. Data are mean±SEM, median [IQR] or baseline-corrected mean difference±SEM. **p<0.01, and ***p<0.001. At week 8, minimally 1 out of 14 glucose measurements was ≤3.9 mmol/L (but not lower than 3.4 mmol/L) in 2 patients on insulin glulisine and in 6 on lixisenatide (a total of 25 out of 504 measurements were ≥3.4 and ≤3.9 mmol/L). From 20 minutes after the meal and onwards, only 1 patient on iGlu and 3 on lixisenatide had minimally 1 measurement in that range (10 out of 340 measurements).

HR and BP by directly augmenting sympathetic outflow from the central nervous system.[19,41] However, although lixisenatide increased HR, the drug decreased SVI and did not affect CI or SNS-activity. This is in accordance with a direct effect of GLP-1RA on the sinoatrial node, as previously suggested,[9,42] whereas SNS-activation would be expected to lead to increased HR, SVI and CI. Second, although the similar HbA_{1c} lowering in both treatment-arms allowed for a clinically relevant comparison, one could speculate that postbreakfast glucose reduction with lixisenatide may have induced a vasoconstrictive response by activating the SNS. However, the number of glucose measurements in the hypoglycaemic range was low and changes in glucose AUC did not explain postbreakfast changes in SBP or DBP. Moreover, SNS-activity did not change. Third, reduced postbreakfast insulin levels with lixisenatide because of inhibition of GER may have reduced insulin-dependent vasodilation[16] and/or increased vascular stiffness.[20] However, although AIX@75 –to a minor extent– explained SBP, we were unable to identify a contributory role of insulin in our exploratory analyses. Notably, insulin resistance may have reduced the vasoactive effects of insulin in our population.[43] Finally, although GLP-1 and GLP-1RA generally have either neutral or vasodilating properties,[7,39] we cannot exclude direct vasoconstrictive properties of lixisenatide, as was also suggested for acute exenatide–infusion in a recent trial in T2DM patients.[9]

Our study has several limitations apart from those discussed above. First, a noninvasive arterial BP monitoring device was used for determination of CI, although this is well validated against intra-arterial measurements.[14] Second, potential study drug-induced alterations in vascular tone may have affected estimations of CI, SVI and SVRI from the finger arterial waveform. Third, AIX is an indirect marker of arterial stiffness. Fourth, we did not specifically measure MSNA. Fifth, inherently to the linear mixed model technique, the overall effect of repeated measurements after the breakfast does not take the amount of time between separate measurements into account.

Perspectives

Here, we demonstrate that 8-week treatment with the short-acting GLP-1RA lixisenatide compared with once-daily titrated iGlu does not affect fasting, but increases postbreakfast BP in insulin glargine-treated overweight T2DM patients. This effect could, at least in-part, be explained by reduced passage rate of nutrients and water, and activation of the gastrovascular reflex. Future studies should establish whether this response influences cardiovascular risk in individual patients or could aid in the prevention of postprandial hypotension.

References

1. Smits MM, Tonneijck L, Muskiet MH, Kramer MH, Cahen DL, van Raalte DH. Gastrointestinal actions of glucagon-like peptide-1-based therapies: glycaemic control beyond the pancreas. *Diabetes Obes Metab* 2016; 18(3): 224-35.
2. Drucker DJ. The Cardiovascular Biology of Glucagon-like Peptide-1. *Cell Metab* 2016; 24(1): 15-30.
3. Asmar A, Simonsen L, Asmar M, et al. Renal extraction and acute effects of glucagon-like peptide-1 on central and renal hemodynamics in healthy men. *Am J Physiol Endocrinol Metab* 2015; 308(8): E641-9.
4. Berkelaar M, Eekhoff EM, Simonis-Bik AM, et al. Effects of induced hyperinsulinaemia with and without hyperglycaemia on measures of cardiac vagal control. *Diabetologia* 2013; 56(6): 1436-43.
5. Bharucha AE, Charkoudian N, Andrews CN, et al. Effects of glucagon-like peptide-1, yohimbine, and nitrergic modulation on sympathetic and parasympathetic activity in humans. *Am J Physiol Regul Integr Comp Physiol* 2008; 295(3): R874-80.
6. Edwards CM, Todd JF, Ghatei MA, Bloom SR. Subcutaneous glucagon-like peptide-1 (7-36) amide is insulinotropic and can cause hypoglycaemia in fasted healthy subjects. *Clin Sci (Lond)* 1998; 95(6): 719-24.
7. Skov J, Pedersen M, Holst JJ, et al. Short-term effects of liraglutide on kidney function and vasoactive hormones in type 2 diabetes: a randomized clinical trial. *Diabetes Obes Metab* 2016; 18(6): 581-9.
8. Tonneijck L, Smits MM, Muskiet MH, et al. Acute renal effects of the GLP-1 receptor agonist exenatide in overweight type 2 diabetes patients: a randomised, double-blind, placebo-controlled trial. *Diabetologia* 2016; 59(7): 1412-21.
9. Smits MM, Tonneijck L, Muskiet MH, et al. Heart rate acceleration with GLP-1 receptor agonists in type 2 diabetes patients: an acute and 12-week randomised, double-blind, placebo-controlled trial. *Eur J Endocrinol* 2017; 176(1): 77-86.
10. Lovshin JA, Zinman B. Blood pressure-lowering effects of incretin-based diabetes therapies. *Can J Diabetes* 2014; 38(5): 364-71.
11. Sun F, Wu S, Guo S, et al. Impact of GLP-1 receptor agonists on blood pressure, heart rate and hypertension among patients with type 2 diabetes: A systematic review and network meta-analysis. *Diabetes Res Clin Pract* 2015; 110(1): 26-37.
12. Muskiet MHA, Tonneijck L, Smits MM, et al. GLP-1 and the kidney: from physiology to pharmacology and outcomes in diabetes. *Nat Rev Nephrol* 2017; 13(10): 605-28.
13. Trahair LG, Horowitz M, Stevens JE, et al. Effects of exogenous glucagon-like peptide-1 on blood pressure, heart rate, gastric emptying, mesenteric blood flow and glycaemic responses to oral glucose in older individuals with normal glucose tolerance or type 2 diabetes. *Diabetologia* 2015; 58(8): 1769-78.
14. Smits MM, Tonneijck L, Muskiet MH, et al. The effects of GLP-1 based therapies on postprandial haemodynamics: Two randomised, placebo-controlled trials in overweight type 2 diabetes patients. *Diabetes Res Clin Pract* 2017; 124: 1-10.
15. Rossi P, Andriesse GI, Oey PL, Wieneke GH, Roelofs JM, Akkermans LM. Stomach distension increases efferent muscle sympathetic nerve activity and blood pressure in healthy humans. *J Neurol Sci* 1998; 161(2): 148-55.
16. Trahair LG, Horowitz M, Jones KL. Postprandial hypotension: a systematic review. *J Am Med Dir Assoc* 2014; 15(6): 394-409.
17. van Orshoven NP, Oey PL, van Schelven LJ, Roelofs JM, Jansen PA, Akkermans LM. Effect of gastric distension on cardiovascular parameters: gastrovascular reflex is attenuated in the elderly. *J Physiol* 2004; 555(Pt 2): 573-83.
18. Thazhath SS, Marathe CS, Wu T, et al. Acute effects of the glucagon-like peptide-1 receptor agonist, exenatide, on blood pressure and heart rate responses to intraduodenal glucose infusion in type 2 diabetes. *Diab Vasc Dis Res* 2017; 14(1): 59-63.
19. Yamamoto H, Lee CE, Marcus JN, et al. Glucagon-like peptide-1 receptor stimulation increases blood pressure and heart rate and activates autonomic regulatory neurons. *J Clin Invest* 2002; 110(1): 43-52.

20. Greenfield JR, Samaras K, Chisholm DJ, Campbell LV. Effect of postprandial insulinemia and insulin resistance on measurement of arterial stiffness (augmentation index). *Int J Cardiol* 2007; 114(1): 50-6.
21. Uetani E, Tabara Y, Igase M, et al. Postprandial hypertension, an overlooked risk marker for arteriosclerosis. *Atherosclerosis* 2012; 224(2): 500-5.
22. Pickering TG, Hall JE, Appel LJ, et al. Recommendations for blood pressure measurement in humans and experimental animals: part 1: blood pressure measurement in humans: a statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. *Circulation* 2005; 111(5): 697-716.
23. Chen CH, Nevo E, Fetics B, et al. Estimation of central aortic pressure waveform by mathematical transformation of radial tonometry pressure. Validation of generalized transfer function. *Circulation* 1997; 95(7): 1827-36.
24. Pauca AL, O'Rourke MF, Kon ND. Prospective evaluation of a method for estimating ascending aortic pressure from the radial artery pressure waveform. *Hypertension* 2001; 38(4): 932-7.
25. Pfeffer MA, Claggett B, Diaz R, et al. Lixisenatide in Patients with Type 2 Diabetes and Acute Coronary Syndrome. *N Engl J Med* 2015; 373(23): 2247-57.
26. Meier JJ, Rosenstock J, Hincelin-Mery A, et al. Contrasting Effects of Lixisenatide and Liraglutide on Postprandial Glycemic Control, Gastric Emptying, and Safety Parameters in Patients With Type 2 Diabetes on Optimized Insulin Glargine With or Without Metformin: A Randomized, Open-Label Trial. *Diabetes Care* 2015; 38(7): 1263-73.
27. Cardoso CRL, Leite NC, Salles GC, Ferreira MT, Salles GF. Aortic stiffness and ambulatory blood pressure as predictors of diabetic kidney disease: a competing risks analysis from the Rio de Janeiro Type 2 Diabetes Cohort Study. *Diabetologia* 2018; 61(2): 455-65.
28. Nurnberger J, Keflioglu-Scheiber A, Opazo Saez AM, Wenzel RR, Philipp T, Schafers RF. Augmentation index is associated with cardiovascular risk. *J Hypertens* 2002; 20(12): 2407-14.
29. Marso SP, Daniels GH, Brown-Frandsen K, et al. Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med* 2016; 375(4): 311-22.
30. Marso SP, Bain SC, Consoli A, et al. Semaglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes. *N Engl J Med* 2016; 375(19): 1834-44.
31. Holman RR, Bethel MA, Mentz RJ, et al. Effects of Once-Weekly Exenatide on Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med* 2017; 377(13): 1228-39.
32. Smits MM, Muskiet MH, Tushuizen ME, et al. Uncomplicated human type 2 diabetes is associated with meal-induced blood pressure lowering and cardiac output increase. *Diabetes Res Clin Pract* 2014; 106(3): 617-26.
33. Jones KL, Rigda RS, Buttfield MDM, Hatzinikolas S, Pham HT, Marathe CS, Wu T, Lange K, Trahair LG, Rayner CK, Horowitz M. Acute effects of lixisenatide on gastric emptying, glycaemia and 'postprandial' blood pressure responses to an oral glucose load in healthy subjects and type 2 diabetes. *Diabetologia.* 2017;60(Suppl 1):S381-382.
34. Trahair LG, Horowitz M, Rayner CK, et al. Comparative effects of variations in duodenal glucose load on glycemic, insulinemic, and incretin responses in healthy young and older subjects. *J Clin Endocrinol Metab* 2012; 97(3): 844-51.
35. Jones KL, Tonkin A, Horowitz M, et al. Rate of gastric emptying is a determinant of postprandial hypotension in non-insulin-dependent diabetes mellitus. *Clin Sci (Lond)* 1998; 94(1): 65-70.
36. Cariga P, Mathias CJ. Haemodynamics of the pressor effect of oral water in human sympathetic denervation due to autonomic failure. *Clin Sci (Lond)* 2001; 101(3): 313-9.
37. Jordan J, Shannon JR, Black BK, et al. The pressor response to water drinking in humans: a sympathetic reflex? *Circulation* 2000; 101(5): 504-9.
38. Shannon JR, Diedrich A, Biaggioni I, et al. Water drinking as a treatment for orthostatic syndromes. *Am J Med* 2002; 112(5): 355-60.
39. Trahair LG, Horowitz M, Hausken T, Feinle-Bisset C, Rayner CK, Jones KL. Effects of exogenous glucagon-like peptide-1 on the blood pressure, heart rate, mesenteric blood flow, and glycemic responses to intraduodenal glucose in

healthy older subjects. *J Clin Endocrinol Metab* 2014; 99(12): E2628-34.

40. Mendis B, Simpson E, MacDonald I, Mansell P. Investigation of the haemodynamic effects of exenatide in healthy male subjects. *Br J Clin Pharmacol* 2012; 74(3): 437-44.
41. Ussher JR, Drucker DJ. Cardiovascular biology of the incretin system. *Endocr Rev* 2012; 33(2): 187-215.
42. Baggio LL, Ussher JR, McLean BA, et al. The autonomic nervous system and cardiac GLP-1 receptors control heart rate in mice. *Mol Metab* 2017; 6(11): 1339-49.
43. Sarafidis PA, Bakris GL. Review: Insulin and endothelin: an interplay contributing to hypertension development? *J Clin Endocrinol Metab* 2007; 92(2): 379-85.

Supplemental methods

Randomisation and intervention

Patients were randomised to lixisenatide or iGlu in a 1:1 ratio. Lixisenatide (Sanofi Aventis, Paris, France) 10 μg was administered for the first 2 weeks, which was subsequently increased to the 20 μg once-daily maintenance dose, injected subcutaneously ~30 minutes before breakfast. The starting dose of iGlu (Sanofi Aventis, Frankfurt am Main, Germany) was 2 units/day once-daily, injected <15 minutes before breakfast, which was self-titrated to the 2-h post-breakfast glucose target ≥5.0 and ≤8.0 mmol/L, according to a predefined altorithm.[1]

Endpoint measurements

Cardiovascular autonomic nerve function

Autonomic function was assessed in the fasting state at Week-0 by standardised cardiovascular reflex tests.[2] Parasympathetic function was evaluated by heart rate variation during deep breathing (maximum-minimum heart rate), as well as the heart-rate response to the Valsalva manoeuvre (Valsalva ratio) and standing (30:15 ratio). Sympathetic function was assessed by the fall in SBP in response to standing. Each result was scored as 0=normal, 1=borderline and 2=abnormal according to predefined criteria[2] for a total maximum score of 8. A score of ≥4 was considered to indicate autonomic dysfunction.

Body impedance analysis

Body fat percentage was measured in the fasting state, while body water percentage was measured before and ~120 minutes after the meal, directly after bladder emptying, using single-frequency bioelectrical impedance (BF-906, Maltron International Ltd, Essex, UK).

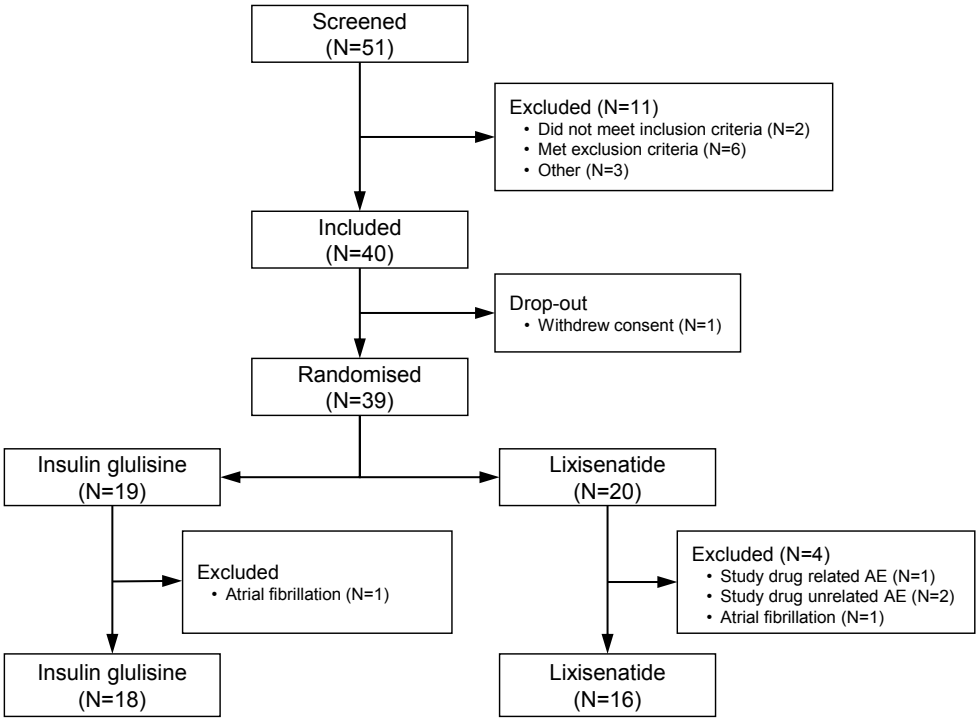
Laboratory analyses

HbA_{1c}, plasma glucose, hematocrit and creatinine were assayed by conventional methods.[1] Blood glucose was determined using a YSI-2300 STAT Glucose analyser (YSI Life Sciences, Yellow Springs, OH). Acetaminophen was measured using an enzymatic immunoassay (Seksisui Diagnostics, Allington, Maidstone, UK) on an Architect 4000 analyser (Abbott Laboratories, Abbott Park, IL). Insulin (endogenous and insulin glargine, but not iGlu)[1] and C-peptide were determined using immunometric assays (Advia Centaur XP, Siemens Medical Solutions Diagnostics, Malvern, PA). PRC was determined with an immunoradiometric kit (Renin III; Cisbio, Gif-sur-Yvette, France). Angiotensin-II was measured by radioimmunoassay after SepPak extraction.[3] Aldosterone was determined by solid-phase radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA). Catecholamines were measured by a high-performance liquid chromatography separation method combined with fluorimetic detection.[4]

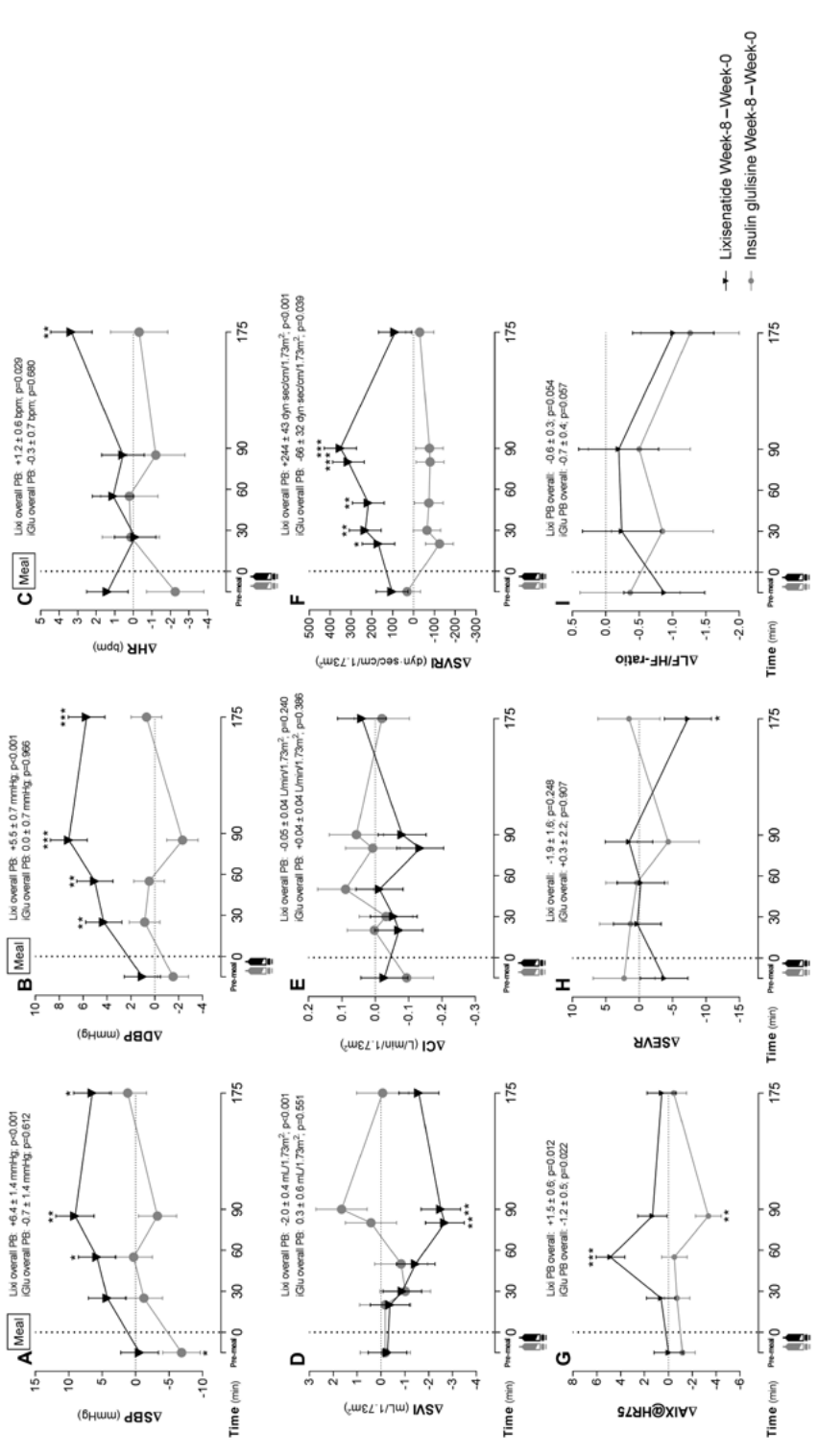
References

1. Tonneijck L, Muskiet MHA, Smits MM, Hoekstra T, Kramer MHH, Danser AHJ, Diamant M, Joles JA, van Raalte DH. Postprandial renal haemodynamic effect of lixisenatide vs once-daily insulin-glulisine in patients with type 2 diabetes on insulin-glargine: An 8-week, randomised, open-label trial. *Diabetes Obes Metab*. 2017;19:1669-1680
2. Ewing DJ, Clarke BF. Diagnosis and management of diabetic autonomic neuropathy. *Br Med J (Clin Res Ed)*. 1982;285:916-918
3. Balcarek J, Seva Pessoa B, Bryson C, Azizi M, Menard J, Garrelds IM, McGeehan G, Reeves RA, Griffith SG, Danser AH, Gregg R. Multiple ascending dose study with the new renin inhibitor vtp-27999: Nephrocentric consequences of too much renin inhibition. *Hypertension*. 2014;63:942-950
4. Willemsen JJ, Ross HA, Jacobs MC, Lenders JW, Thien T, Swinkels LM, Benraad TJ. Highly sensitive and specific hplc with fluorometric detection for determination of plasma epinephrine and norepinephrine applied to kinetic studies in humans. *Clin Chem*. 1995;41:1455-1460

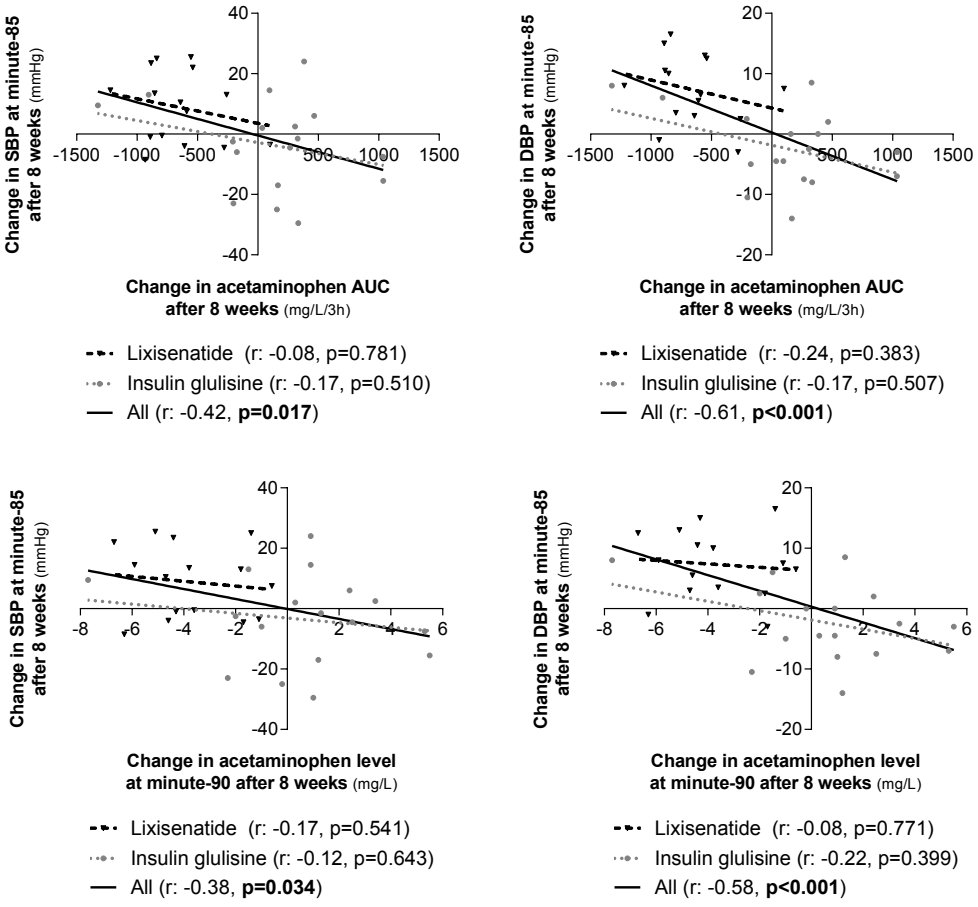
Supplemental data



Supplemental Figure 1. Flow diagram of study participants



Supplemental Figure 2. Within-group differences of 8-week treatment with lixisenatide or insulin glulisine. Data are mean difference ± SEM using linear mixed models to examine lixisenatide or insulin glulisine-induced effects compared to baseline. * p<0.05, ** p<0.01, and *** p<0.001. AIX@HR75, augmentation index normalised to heart rate of 75 bpm; CI, cardiac index; DBP, diastolic blood pressure; LF/HF-ratio, ratio of low-frequency to high frequency; PB, post-breakfast. SBP, systolic blood pressure; SVI, stroke volume index; SVRI, systemic vascular resistance index.



Supplemental Figure 3. Correlations. Spearman signed rank test was used to assess correlations.

Supplemental Table 1. Responses in metabolic measurements, body weight and composition, RAAS hormones and catecholamines

	Insulin glulisine (N=18)		
Variables	**Week-0**	**Week-8**	**Within-group P-value**
Metabolic measurements			
HbA_{1c}, %	7.8±0.2	7.2±0.1	**0.001**
HbA_{1c}, mmol/mol	62±9	55±7	**<0.001**
Fasting plasma glucose, mmol/L	7.1 [5.9-8.2]	7.3 [6.4-7.9]	0.975
Insulin AUC, nmol/L/3h	67.0 [50.8-126.6]	66.0 [50.5-122.5]	0.733
C-peptide AUC, nmol/L/3h	236 [126-355]	169 [114-279]	**0.008**
Body weight and composition			
Body weight, kg	95.8±4.2	96.6±4.3	0.119
Waist circumference, cm	115.5 [107.0-122.1]	115.5 [107.1-124.3]	**0.027**
Hip circumference, cm	112.0 [107.8-116.4]	113.0 [105.8-118.3]	0.379
Waist/hip ratio	1.01±0.02	1.02±0.02	0.619
Fasting body fat, %	37.0 [33.0-43.4]	36.5 [32.5-43.7]	0.910
Fasting body water, %	46.9 [42.1-49.3]	46.5 [41.6-49.6]	0.401
Post-breakfast body water, %	46.5 [42.4-48.8]	46.8 [42.8-49.1]	0.311
Fasting hematocrit, L/L	0.41±0.01	0.40±0.01	**0.035**
Post-breakfast hematocrit, L/L	0.41±0.01	0.40±0.01	0.287
RAAS hormones			
Fasting PRC, pg/mL	11.4 [7.3-15.5]	10.5 [6.9-14.9]	0.502
Post-breakfast PRC, pg/mL	11.4 [6.1-13.6]	10.7 [6.9-15.0]	0.874
Fasting Angiotensin-II, fmol/mL	8.0 [5.9-9.8]	8.0 [6.5-10.4]	0.586
Post-breakfast angiotensin-II, fmol/mL	7.7 [5.1-8.9]	8.9 [6.3-10.5]	0.076
Fasting Aldosterone, pg/mL	47.9 [30.8-88.2]	44.5 [32.8-73.1]	0.112
Post-breakfast aldosterone, pg/mL	33.9 [24.7-48.5]	27.4 [17.5-51.1]	0.396
Catecholamines			
Post-breakfast noradrenaline, nmol/L	1.74±0.13	1.86±0.18	0.274
Post-breakfast adrenaline, nmol/L	0.08±0.01	0.08±0.01	0.439
Post-breakfast dopamine, nmol/L	0.08±0.01	0.08±0.01	0.869

Data are mean ± SEM, median [IQR] or baseline-corrected mean difference (SEM) using multiple linear regression to examine baseline-corrected lixisenatide-induced effects compared to insulin glulisine. Paired t-tests or Wilcoxon signed rank tests were used for within-group comparisons. Significant differences indicated in bold font. §indicates baseline-corrected ratio ± SEM using

Lixisenatide (N=16)				
Week-0	**Week-8**	**Within-group P-value**	Mean ± SEM difference lixi-iGluli	p-value
8.4±0.2	7.6±0.2	**<0.001**	0.0±0.2	0.946
68±9	60±9	**<0.001**	0±2	0.870
6.6 [4.9-8.4]	6.1 [5.0-8.0]	0.500	-0.7±0.57	0.265
57.0 [41.8-81.1]	47.5 [39.2-65.1]	**0.003**	-11.6±6.7	0.095
189 [145-239]	130 [89-192]	**0.001**	-9±18	0.637
99.6±3.9	99.1±3.9	0.074	-1.4±0.6	**0.022**
109.5 [105.5-118.1]	109.5 [106.6-119.1]	0.459	-1.2±0.9	0.184
111.5 [109.6-115.4]	110.8 [107.5-117.1]	0.154	-1.5±1.1	0.179
1.00±0.02	1.00±0.02	0.323	0.0±0.0	0.942
32.7 [28.6-39.8]	32.1 [29.0-38.4]	0.955	-0.6±0.6	0.290
49.3 [44.1-52.3]	49.6 [45.1-51.9]	0.932	0.7±0.4	0.082
47.0 [43.9-51.6]	46.8 [44.2-51.2]	0.753	-0.5±0.4	0.274
0.40±0.01	0.39±0.01	**0.039**	0.00±0.01	0.850
0.41±0.01	0.41±0.01	0.281	0.01±0.01	**0.045**
8.8 [3.3-32.6]	10.5 [3.2-24.9]	0.554	0.76±1.26[§]	0.242
8.3 [3.5-30.1]	8.7 [2.7-22.6]	0.113	0.61±1.26[§]	**0.041**
6.9 [3.8-8.3]	8.6 [6.9-11.4]	**0.041**	1.05±0.99	0.300
7.0 [3.9-8.6]	8.4 [6.6-9.5]	0.163	0.02±1.02	0.983
50.7 [32.1-88.4]	45.2 [24.7-79.7]	0.121	0.08±6.37	0.990
29.2 [22.1-48.6]	47.2 [25.4-65.2]	0.196	10.64±7.77	0.181
1.57±0.12	1.66±0.11	0.516	-0.06±0.16	0.697
0.10±0.02	0.08±0.01	0.097	-0.01±0.01	0.565
0.07±0.00	0.07±0.01	0.798	0.89±1.10	0.246

multiple linear regression. Abbreviations: AUC, are under the curve; PRC, plasma renin concentration; RAAS, renin-angiotensin-aldosterone-system.