Heart rate acceleration with GLP-1 receptor agonists in type 2 diabetes patients: an acute and 12-week randomised, double-blind, placebo-controlled trial

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Abstract

Objective: To examine mechanisms underlying resting heart rate (RHR) increments of GLP-1 receptor agonists in type 2 diabetes patients.

Design: Acute and 12-week randomised, placebo-controlled, double-blind, single-centre, parallel-group trial.

Methods: In total, 57 type 2 diabetes patients (mean ± s.d. age: 62.8 ± 6.9 years; BMI: 31.8 ± 4.1 kg/m²; HbA1c: 7.3 ± 0.6%), treated with metformin and/or sulfonylureas, were included between July 2013 and August 2015. In the acute study, the GLP-1 receptor agonist exenatide (n = 29) or placebo (saline 0.9%; n = 28) was infused intravenously. Subsequently, patients were again randomised to receive the GLP-1 receptor agonist liraglutide (n = 19) or matching placebo (n = 17) for 12 weeks. RHR and blood pressure (BP) were measured by oscillometric technique, systemic haemodynamics by finger photoplethysmography, sympathetic nervous system (SNS) activity by heart rate variability and arterial stiffness by applanation tonometry. This trial was registered at ClinicalTrials.gov (NCT01744236).

Results: Exenatide-infusion increased RHR (mean ± s.e.m. +7.5 ± 0.9 BPM, P < 0.001), and systolic and diastolic BP (both P < 0.05), compared with placebo. Vascular resistance increased during exenatide-infusion, whereas stroke volume and arterial stiffness decreased (P < 0.05). SNS activity and cardiac output were unaffected. Twelve-week treatment with liraglutide increased RHR (+6.6 ± 2.1 BPM), while reducing systolic BP (−12.6 ± 4.7 mmHg) and stroke volume (all P < 0.01). Cardiac output, vascular resistance, arterial stiffness and SNS activity remained unchanged (all P > 0.05).

Conclusions: RHR acceleration with acute and 12-week GLP-1 receptor agonist treatment in type 2 diabetes patients is not explained by changes in SNS activity, and our data argue against vasodilation. In line with pre-clinical data, direct sino-atrial stimulation may be involved.

Introduction

Glucagon-like peptide (GLP)-1 receptor agonists are frequently used for the management of hyperglycaemia in type 2 diabetes patients. These antihyperglycaemic agents not only improve pancreatic islet-cell function but also display various extra-pancreatic effects, which include actions on the cardiovascular system (1).
Although physiological levels of GLP-1 do not affect resting heart rate (RHR) (2), infusion of GLP-1 peptide to supraphysiological levels and GLP-1 receptor agonist treatment increases RHR (1). This effect occurs within hours (3) and sustains during prolonged administration in type 2 diabetes patients (4). An acceleration of ~2 beats/min is regularly observed, yet some studies report increases of over ~10 beats/min (4, 5). GLP-1 receptor agonists with a long half-life (‘long-acting’ agents) demonstrate a sustained RHR throughout the day, whereas with short-acting agents, this increase is transient (5, 6). Nevertheless, with 24-h measurements, an overall slight increase in RHR is also observed with short-acting agents (5, 6).

Elevated RHR has been associated with all-cause mortality in large epidemiological trials (7). Although some suggest that RHR simply reflects general fitness, and may by itself not be a risk factor for mortality, others suggest that RHR elevation independently causes or aggravates atherosclerosis and increases myocardial ischaemia (8). As such, RHR accelerating effects of GLP-1 receptor agonists could potentially lead to untoward effects. Long-term cardiovascular safety trials that study this antihyperglycaemic drug class are currently ongoing; however, it can be debated whether the average follow-up time of these trials is sufficient to assess adverse consequences of RHR acceleration, which will likely take years to develop (8, 9).

The mechanisms underlying the RHR acceleration are not fully understood. Based on experiments in animal models of type 2 diabetes and small-sized studies in healthy volunteers (3, 10, 11), several hypotheses have been proposed and tested. First, GLP-1 receptor agonists may directly stimulate the sympathetic nervous system (SNS) (11). Second, the RHR increment may be caused by baroreflex activation, secondary to vasodilatation of arterial (resistance) vessels and subsequent reduction in systemic vascular resistance (SVR) (10). Finally, direct effects on sino-atrial cells have been suggested (5). Unfortunately, the results of these studies are conflicting, and the exact mechanism(s) remain unclear. Moreover, current evidence is incomplete as studies have only been performed in either an acute or prolonged intervention setting, whereas the effects of GLP-1 receptor agonists may differ over time, as has been demonstrated for their effects on blood pressure (BP) and gastric emptying (3, 4, 12). Also, hitherto, no studies have integrally assessed these potential mechanisms in type 2 diabetes patients. Therefore, the primary aim of the current study was to assess the mechanisms underlying the RHR acceleration with the use of GLP-1 receptor agonists in type 2 diabetes patients, both in the acute setting and after 12-week treatment.

### Subjects and methods

In the current study, patients with type 2 diabetes underwent two randomised, placebo-controlled, double-blind trials, at the VU University Medical Center, as described elsewhere (13). In short, after inclusion and a run-in period of 4 weeks, baseline measurements for both the acute and 12-week intervention study were obtained (Supplementary Fig. A, see section on supplementary data given at the end of this article). Then, the acute effects of the GLP-1 receptor agonist exenatide (AstraZeneca) vs placebo (saline 0.9%) were assessed. Subsequently, the placebo-controlled effects of 12-week treatment with the GLP-1 receptor agonist liraglutide (Novo Nordisk A/S) or DPP-4 inhibitor sitagliptin (Merck & Co) were studied. Importantly, as DPP-4 inhibitors have no documented effect on RHR in previous studies, the results of this treatment arm are only provided in the Online appendix.

The study was approved by the ethics review board of the VU University Medical Center, was registered at ClinicalTrials.gov (ID: NCT01744236) and was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization of Good Clinical Practice. All patients provided written informed consent before participation.

### Participants

We recruited 60 Caucasian male and (post-menopausal) female patients with type 2 diabetes. Inclusion criteria were ages between 35 and 75 years, BMI of 25–40 kg/m², HbA₁c of 6.5–9.0% (48–75 mmol/mol) and treatment with a stable dose of metformin and/or sulphonylurea derivative for at least 3 months. Exclusion criteria were active or history of pancreatic disease or malignancy, active hepatic disease or renal impairment (estimated glomerular filtration rate <60 mL/min/1.73 m²) and autonomic neuropathy assessed by Ewing tests (14). Moreover, patients with a recent (<6 months) history of cardiovascular disease, including acute coronary syndrome, cerebrovascular disorders, heart failure or atrial fibrillation, were excluded. Patients were not treated with insulin- or GLP-1-based therapy at the time of inclusion. Apart from the use of diuretics, use of antihypertensive medication was not an exclusion criterion (13).
Randomisation and treatment

Randomisation was performed by the trial pharmacist using computer-generated numbers. Stratification for the acute intervention study (block size of 6; allocation ratio of 1:1) was applied to have equal numbers among the groups for the 12-week study (block size of 6; allocation ratio of 1:1:1).

For the acute study, exenatide or placebo was administered intravenously, allowing us to perform a blinded study in the absence of exenatide placebo-pens for subcutaneous administration. A loading dose of 50 ng/min for 30 min, followed by a continuous infusion of 25 ng/min, rapidly yields stable plasma levels within the therapeutic range and harbours a good tolerability profile (15). Importantly, similar to subcutaneous use, intravenous exenatide increases RHR as demonstrated previously in healthy volunteers (3, 10).

For the 12-week study, liraglutide was chosen as (1) the once-daily administration may promote therapy adherence and (2) visually identical pre-filled pens with liraglutide or placebo were provided by Novo Nordisk A/S, allowing a double-blinded study. All patients received pre-filled pens for subcutaneous injections (containing liraglutide or placebo) and oral capsules (containing sitagliptin 100 mg or placebo). ACE Pharmaceuticals (Zeewolde, The Netherlands) encapsulated sitagliptin or placebo. Study drugs were administered once-daily in the evening. For the subcutaneous injections, a dose-increment schedule was used (week 1: 0.6 mg daily; week 2: 1.2 mg daily; week 3 and remaining weeks: 1.8 mg daily). Based on tolerance to the study drug, time between dose increments could be extended and drug dose could be reduced, based on the investigators’ discretion.

Endpoint measurements

Prior to the test visits, patients adhered to an average sodium chloride (9–12 g/day) and protein (1.5–2.0 g/kg/day) diet for two days. Moreover, as of 24 h before the visit, patients abstained from heavy physical exercise, alcohol and caffeine. After an overnight fast, patients refrained from taking their morning medication, apart from metformin and thyroid hormone replacement therapy. Prior to each measurement, patients were acclimatised for at least 10 min. All measurements were performed in the fasting state, at the non-dominant arm comfortably placed at heart level, in a semi-recumbent position in a temperature-controlled room (23.0 ± 1.0°C). Appropriate cuff sizes were used where applicable.

Haemodynamic assessments

An automatic oscillometric device (Dinamap, GE Healthcare) was used to measure systolic blood pressure (SBP) and diastolic blood pressure (DBP), mean arterial pressure (MAP) and HR. Measurements were performed in triplicate at 1- to 2-min intervals, the mean of the last 2 measurements was used for each time point. A non-invasive beat-to-beat finger arterial blood pressure monitoring device (Nexfin, BM Eye, Amsterdam, The Netherlands) was used to calculate stroke volume (SV), cardiac output (CO) and systemic vascular resistance (SVR) from the obtained pulse waveform. SV, CO and SVR were normalised for body surface area, to obtain SV index (SVI), cardiac index (CI) and SVR index (SVRI) respectively. Finger plethysmographic measurements were performed over a period of 30 s, and an average was derived using dedicated software (Nexfin®PC version 2, BM Eye). The within-day and between-day variability of these measurements are ≤9.1%, based on the data from a previous study in healthy volunteers (3).

Heart rate variability (HRV) assessments

Using an ECG-equipped Nexfin device, 5-min recordings were made in the resting state, during which participants were instructed to breathe spontaneously (range 10–18 breaths/min) and refrain from speaking or sleeping. ECG measurements were visually inspected and artefacts were manually corrected, using linear interpolation when necessary. ECG strips were entered into Kubios HRV Analysis Software 2.1 (University of Eastern Finland, Kuopio, Finland). After further automated artefact correction and removal of trend components, Fast Fourier spectral analyses were performed to measure cardiac autonomic nervous system (ANS) balance. The derived spectrum included the low-frequency (LF; 0.04–0.15 Hz) and high-frequency (HF; 0.15–0.5 Hz) bands, from which the LF/HF ratio, a validated marker for sympathovagal ANS balance, was calculated and used for the current analysis (16). Additionally, corrected ECG R-R signals were analysed to measure the standard deviation of the successive heart beats (SDNN) and the root mean squares of the successive differences between adjacent heart beats (RMSSD), which are predictors of cardiovascular mortality (16).

Pulse wave analysis

Pulse wave analysis (PWA) was performed with a SphygmoCor applanation tonometry system.
(AtCor Medical Systems Inc., West Ryde, Sydney, Australia) to assess the arterial stiffness at the level of the radial artery. We used the average of 2 recordings (of ≥12 s), which were performed at the radial artery and were required to have adequate pulse wave profiles and good quality control (defined as a quality index of >80%). The augmentation index (an estimate of vascular compliance) was calculated as the difference between the second systolic peak and inflection point, expressed as a percentage of the central pulse pressure corrected for an average HR of 75 beats/min (AIX@HR75). Moreover, the subendocardial viability ratio (SEVR), an index of myocardial perfusion relative to left ventricular workload, was determined.

**Body impedance analysis**

Body water percentage was assessed using the single-frequency bioelectrical impedance analyser (BIA) Maltron BF-906 (Maltron International Ltd, Essex, UK). Measurements were taken directly after bladder emptying: at baseline, two hours after start of the acute intervention and after the 12-week intervention.

**Laboratory measurements**

Blood samples were drawn from an intravenous catheter placed in an antecubital vein of the non-dominant forearm. Venous plasma glucose was assessed with an YSI 2300 STAT Glucose analyser (YSI Life Sciences, Yellow Springs, OH, USA). Haematocrit was measured using the automated Cell-Dyn Sapphire (Abbott Diagnostics). Insulin was determined from serum using an immunometric assay (Advia Centaur XP Immunoassay System, Siemens Healthcare).

**Study end points**

The primary end point of this study was liraglutide-induced change in LF/HF ratio from baseline to 12 weeks of treatment, compared with placebo (13). This primary endpoint was chosen because an increase in SNS activity was deemed the most probable explanation for RHR-elevation with GLP-1 receptor agonists based on animal data and small clinical studies (13). All other parameters, as measured at baseline, and after acute intervention or 12-week treatment, were considered as secondary end points. Effects on RHR and BP were additionally measured after 2 and 6 weeks of treatment, to assess the potential time-dependent effects.

**Statistics**

The sample size justification for this study has been described previously (13). In short, 20 patients were needed to be included per treatment arm (i.e. acute exenatide or placebo or 12-week liraglutide, sitagliptin or placebo), taking into account a drop-out rate of 15%. All data were double-entered into an electronic data management system (OpenClinica LLC, version 3.3, Waltham, MA, USA) and exported to the study database. Data are presented as means ± standard error of the mean (s.e.m.) or, in case of a non-Gaussian distribution, as median (interquartile range). Treatment-induced effects are displayed as the maximal effect at any time during the treatment period, corrected for placebo effects, unless stated otherwise.

To test the treatment effects vs placebo, multivariable regression models (for single measured continuous endpoints) and linear mixed models (LMM, for repeatedly measured continuous endpoints) were used in the per-protocol population. The endpoint of interest was added as dependent variable, and treatment allocation as independent variable; for the 12-week study, treatment with liraglutide or sitagliptin were included as dummy variables. For the multivariable regression models, we additionally included the corresponding baseline

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**Figure 1**

Study flowchart.
Table 1  Baseline characteristics and drug-induced effects. Baseline values and placebo-corrected drug-induced changes for the acute and 12-week intervention study are presented as mean±S.E.M. Baseline values were statistically not different between the treatment groups. For the acute study, the maximum exenatide-induced change was noted; for the long-term study, the change after 12 weeks of treatment was noted. The absolute changes are shown in Fig. 1 for the acute study and in Fig. 2 for the 12-week study.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo</th>
<th>Exenatide</th>
<th>Placebo</th>
<th>Liraglutide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Plac. corr. max. effect</td>
<td>Baseline</td>
<td>Plac. corr. effect</td>
</tr>
<tr>
<td>n</td>
<td>28</td>
<td>29</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>Age (years)</td>
<td>64.3 ± 1.2</td>
<td>61.4 ± 1.4</td>
<td>65.8 ± 1.4</td>
<td>60.5 ± 1.6</td>
</tr>
<tr>
<td>Male sex (n (%))</td>
<td>23 (82.1%)</td>
<td>21 (72.4%)</td>
<td>13 (76.5%)</td>
<td>14 (73.7%)</td>
</tr>
<tr>
<td>Duration of T2D (years)</td>
<td>8.6 ± 1.0</td>
<td>7.3 ± 0.8</td>
<td>8.2 ± 1.2</td>
<td>7.7 ± 1.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>98.8 ± 2.2</td>
<td>101.3 ± 3.6</td>
<td>95.8 ± 2.4</td>
<td>105.9 ± 3.9</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.2 ± 0.7</td>
<td>32.1 ± 0.9</td>
<td>30.6 ± 0.7</td>
<td>33.3 ± 1.0</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.3 ± 0.1</td>
<td>7.3 ± 0.1</td>
<td>7.5 ± 0.2</td>
<td>7.4 ± 0.2</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>56.4 ± 1.5</td>
<td>55.9 ± 1.2</td>
<td>58.2 ± 1.9</td>
<td>57.1 ± 1.7</td>
</tr>
<tr>
<td>Use of (n (%))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metformin</td>
<td>26 (92.9%)</td>
<td>28 (96.6%)</td>
<td>15 (88.2%)</td>
<td>19 (100%)</td>
</tr>
<tr>
<td>Sulphonylurea</td>
<td>13 (46.4%)</td>
<td>12 (41.4%)</td>
<td>8 (47.1%)</td>
<td>7 (36.8%)</td>
</tr>
<tr>
<td>Any anti-RR</td>
<td>17 (60.7%)</td>
<td>21 (72.4%)</td>
<td>11 (64.7%)</td>
<td>15 (78.9%)</td>
</tr>
<tr>
<td>Beta blocker</td>
<td>5 (17.9%)</td>
<td>5 (17.2%)</td>
<td>4 (23.5%)</td>
<td>3 (15.8%)</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>8 (28.6%)</td>
<td>11 (37.9%)</td>
<td>5 (29.4%)</td>
<td>9 (47.4%)</td>
</tr>
<tr>
<td>ARB</td>
<td>8 (28.6%)</td>
<td>9 (31.0%)</td>
<td>6 (35.3%)</td>
<td>6 (31.6%)</td>
</tr>
<tr>
<td>Ca-antagonist</td>
<td>3 (10.7%)</td>
<td>8 (27.6%)</td>
<td>3 (17.6%)</td>
<td>5 (26.3%)</td>
</tr>
<tr>
<td>Endpoints</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RHR (BPM)</td>
<td>66.5 ± 1.9</td>
<td>63.2 ± 1.2</td>
<td>+7.5 ± 0.9***</td>
<td>64.5 ± 2.2</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>13.1 ± 3.0</td>
<td>135.5 ± 2.6</td>
<td>+6.7 ± 3.2*</td>
<td>137.6 ± 3.6</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>76.2 ± 1.2</td>
<td>76.3 ± 1.3</td>
<td>+4.9 ± 1.4***</td>
<td>76.4 ± 1.6</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>97.4 ± 1.9</td>
<td>97.5 ± 1.7</td>
<td>+7.7 ± 1.9***</td>
<td>99.1 ± 2.5</td>
</tr>
<tr>
<td>SVI (ml/1.73 m²)</td>
<td>47.6 ± 1.4</td>
<td>48.2 ± 0.9</td>
<td>−2.9 ± 1.0**</td>
<td>47.4 ± 1.8</td>
</tr>
<tr>
<td>CI (L/min/1.73 m²)</td>
<td>3.1 ± 0.1</td>
<td>2.9 ± 0.1</td>
<td>+0.25 ± 0.08</td>
<td>2.9 ± 0.1</td>
</tr>
<tr>
<td>SVRI (dyn * s/ cm²/1.73 m²)</td>
<td>2516.5 ± 103.2</td>
<td>2615.7 ± 92.1</td>
<td>+238.6 ± 97.2*</td>
<td>2664.0 ± 130.1</td>
</tr>
<tr>
<td>AIX@HR75 (%)</td>
<td>23.1 ± 1.6</td>
<td>22.1 ± 1.4</td>
<td>−2.8 ± 1.3</td>
<td>23.3 ± 1.8</td>
</tr>
<tr>
<td>SEVR (%)</td>
<td>145.0 ± 4.9</td>
<td>146.5 ± 3.5</td>
<td>+15.4 ± 3.2***</td>
<td>146.1 ± 5.1</td>
</tr>
<tr>
<td>LF/HF-ratio</td>
<td>3.3 ± 1.1</td>
<td>1.9 ± 0.3</td>
<td>−0.4 ± 1.1</td>
<td>1.9 ± 0.5</td>
</tr>
<tr>
<td>SDNN</td>
<td>22.9 ± 2.8</td>
<td>22.4 ± 1.5</td>
<td>+4.6 ± 2.1*</td>
<td>22.2 ± 4.5</td>
</tr>
<tr>
<td>RMSSD</td>
<td>22.2 ± 3.9</td>
<td>21.6 ± 1.8</td>
<td>+4.9 ± 2.4*</td>
<td>22.5 ± 6.1</td>
</tr>
</tbody>
</table>

Statistical values for treatment-induced changes are indicated as **P<0.05; ***P<0.01; ****P<0.001.

ACE, angiotensin-converting enzyme; AIX@HR75, augmentation index normalised to heart rate 75 beats per minute; anti-RR, antihypertensive agent; ARB, angiotensin receptor blocker; BPM, beats per minute; Ca antagonist, calcium antagonist; CI, cardiac index; DBP, diastolic blood pressure; MAP, mean arterial pressure; NA, not applicable; RHR, resting heart rate; RMSSD, the root mean squares of the successive differences between adjacent heart beats; SBP, systolic blood pressure; SDNN, standard deviation of the successive heart beats; SEVR, subendocardial viability ratio; SVI, stroke volume index; SVRI, systemic vascular resistance index.

Results

We included 60 type 2 diabetes patients between July 2013 and March 2015. Because three patients withdrew consent before baseline measurements (Fig. 1), 57 patients were enrolled and analysed for the acute study (exenatide n = 29, placebo n = 28). Prior to randomisation for the 12-week intervention study, we excluded one patient because of an incidental finding. One patient in the sitagliptin group discontinued treatment due to adverse effects (pollakiuria/dizziness).
Thus, 55 patients were analysed for the 12-week study (liraglutide n = 19, sitagliptin n = 19, and placebo n = 17). In the liraglutide-group, one patient completed the study using liraglutide 0.6 mg once-daily, as the patient did not tolerate higher doses. Baseline characteristics were generally well balanced between treatment groups (Table 1).

**Acute intervention trial**

**Systemic haemodynamics**

Exenatide increased RHR by $7.5 \pm 0.9$ beats per minute (BPM) ($P < 0.001$), compared with placebo (Fig. 2 for baseline-subtracted values). In 2 patients, a RHR acceleration larger than 15 BPM was observed. Exenatide increased SBP by $6.7 \pm 3.2$ mmHg ($P < 0.05$), DBP by $4.8 \pm 1.4$ mmHg ($P < 0.001$) and MAP by $7.7 \pm 1.9$ mmHg ($P < 0.001$). SVI decreased in the first 80 min after exenatide-infusion (max $2.9 \pm 0.9$ mL/1.73 m$^2$; $P < 0.01$), although no between-group differences were seen after this time point. CI was initially not affected by exenatide, yet increased after 155 min ($P < 0.01$). Exenatide increased SVRI by $238.6 \pm 97.2$ dyn*s/cm$^5$/1.73 m$^2$ ($P < 0.05$). Augmentation index reduced by $-2.8 \pm 1.3\%$ ($P < 0.05$) after exenatide-infusion and SEVR by $-15.4 \pm 3.2\%$ ($P < 0.001$).

**ANS/HRV**

Exenatide-infusion did not affect the LF/HF ratio ($P > 0.05$), although it increased SDNN ($4.6 \pm 2.1$; $P < 0.05$) and RMSSD ($4.9 \pm 2.4$; $P < 0.05$).

**Laboratory measurements and anthropometrics**

Exenatide-infusion decreased blood glucose levels by $1.6 \pm 0.1$ mmol/L ($P < 0.005$; Supplementary Fig. B) and increased insulin levels by $54.9 \pm 9.6$ pmol/L ($P < 0.001$). Exenatide-infusion marginally increased haematocrit by $0.01 \pm 0.004$ L/L ($P < 0.05$), whereas no effect on body water percentage was observed ($P > 0.05$).

**Adverse effects**

In the exenatide-group, 4 patients experienced nausea without vomiting, whereas mild headache and diarrhoea occurred in 1 patient. No adverse events occurred in the placebo group.

**12-week intervention trial**

**Systemic haemodynamics**

RHR increased with $6.6 \pm 2.1$ BPM ($P < 0.01$) at 12 weeks with liraglutide treatment, compared with placebo (Fig. 3 for baseline-subtracted values). Liraglutide treatment reduced SBP by $12.6 \pm 4.7$ mmHg ($P < 0.01$) at 12 weeks and SVI by $4.5 \pm 1.3$ ($P = 0.001$). No effects of liraglutide were seen on DBP, MAP, CI or SVRI ($P > 0.05$). Moreover, liraglutide had no effect on augmentation index or SEVR ($P > 0.05$).

**ANS/HRV**

Liraglutide had no effect on LF/HF ratio, SDNN or RMSSD (all $P > 0.05$).
Laboratory measurements and anthropometrics

Liraglutide decreased HbA1c by 1.7 ± 0.9%–(14.1 ± 2.3 mmol/mol) (P < 0.001) and fasting glucose by 1.7 ± 0.5 mmol/L (P = 0.001; Supplementary Fig. B), compared with placebo, while increasing fasting insulin by 52 ± 11.0 pmol/L (P < 0.001). Liraglutide reduced body weight (1.9 ± 0.7 kg; P = 0.005), but had no effect on haematocrit or body water percentage (P > 0.05).

Adverse effects

Gastrointestinal complaints (nausea and diarrhoea) were present in 12 patients treated with liraglutide and in no patients receiving placebo. Other adverse effects were experienced in equal numbers among all treatment groups.

Sitagliptin-treated arm

Sitagliptin had no effect on any of the tested haemodynamic or autonomic nervous system parameters; results are shown in the Online appendix (Supplementary Fig. C).

Discussion

This is the first combined acute and prolonged intervention study that assesses the mechanisms underlying GLP-1 receptor agonists-associated RHR acceleration in type 2 diabetes patients. Our findings suggest that increases in RHR by GLP-1 receptor agonist treatment are not caused by changes in systemic haemodynamics, vascular resistance/stiffness or SNS activity, making a direct GLP-1 receptor agonist-mediated stimulation of the sino-atrial cells the most likely explanation.

RHR increased after acute exenatide and 12-week liraglutide administration in type 2 diabetes patients, confirming previous clinical studies using GLP-1 receptor agonists (1). Although the divergent pharmacological composition and route of administration of intravenous exenatide and subcutaneous liraglutide hamper a direct comparison of these drugs (17), their effects on RHR were similar, and hint towards a comparable underlying mechanism. Our prime hypothesis was that an increase in sympathetic drive or decrease in parasympathetic activity augmented RHR. Previous trials studying GLP-1 receptor agonists in the acute setting in healthy volunteers suggested a direct stimulation of SNS activity (3, 11), as measured by LF/HF ratio, rate pressure product or muscle nerve activity. Similarly, after 5-week treatment in patients with type 2 diabetes (5), LF/HF ratio increased. However, in the current trials, neither acute exenatide nor long-term liraglutide affected LF/HF ratio. Moreover, SV and CI, which normally increase substantially during SNS activation, were not or only marginally raised (18). Although not currently tested given the small sample size, a previous study observed RHR acceleration in patients.
using beta-blocking agents (19), further arguing against SNS involvement.

A second hypothesis of RHR acceleration is based on reduced vascular resistance, which activates homeostatic baroreflex mechanisms, leading to RHR acceleration to maintain stable tissue perfusion (termed ‘reflex tachycardia’) (10). Notably, the sensitivity of baroreflex mechanisms is closely and inversely linked to arterial stiffness. As such, in subjects with increased arterial stiffness, the HR response to altered BP is lowered (20). Although acute exenatide-infusion increased RHR and decreased arterial stiffness (AIX@ HR75), this was accompanied by increases in SBP, DBP and SVRI, ruling out increased baroreflex activity as a cause of the increase in heart rate. Although BP reduced with 12-week liraglutide treatment, we observed no changes in SVRI or vascular stiffness, suggesting that in this condition, the baroreceptor reflex does not contribute significantly to the effects of GLP-1 receptor agonists on RHR either.

As our data argue against SNS activity or vasodilation as a cause of the GLP-1 receptor agonists-induced RHR acceleration, one hypothesis by exclusion is now more likely: direct stimulation of the sino-atrial node. GLP-1 receptors are localised on sino-atrial node cells in monkey and human tissue (21), suggesting that these receptors may directly affect RHR. Although dedicated experimental data are currently lacking, GLP-1 receptor stimulation in cardiac myocytes tissue increases cyclic-AMP signalling (1), a pathway known to stimulate sino-atrial-mediated RHR acceleration (22). Moreover, direct RHR stimulation is accompanied by decreased diastolic filling time and reduced SVI, thereby keeping CI unaffected (23). This line of reasoning matches our observations in both the acute and 12-week intervention trials.

During acute exenatide-infusion, an increase in SBP and DBP was observed, whereas 12-week liraglutide treatment reduced SBP. This discrepancy between acute and prolonged GLP-1 receptor agonist intervention is known from previous clinical studies in type 2 diabetes (3, 4, 24, 25). Mechanisms underlying these effects remain incompletely understood. Although our study was not dedicated to assess such mechanisms, our data suggest that the acute BP-rising effect might be caused by vasoconstriction (indicated by the increase in SVRI). For the 12-week study, the current study indicates that the GLP-1-induced BP reduction may not be caused by reduced vascular resistance or SNS activity. Dedicated studies are necessary to understand the GLP-1 receptor agonist-induced BP reduction.

RHR acceleration is associated with all-cause mortality in large epidemiological trials (7). It has been suggested that RHR simply reflects general fitness, and as such, is linked with mortality (8). However, others suggest that RHR elevation is an independent risk factor for atherosclerosis and chronic myocardial ischaemia (8). Thus, RHR-accelerating effects of GLP-1 receptor agonists could potentially lead to untoward effects. In the current trials, despite an increase in RHR, which lowers myocardial perfusion time, we observed no changes in SEVR, which estimates myocardial workload and oxygenation (26). HRV, which predicts cardiovascular mortality when reduced (16), increased during exenatide administration and was not affected by liraglutide treatment. However, with only 12 weeks of follow-up, development of atherosclerosis and heart failure could not be determined.

The clinical relevance of potential harmful changes in RHR by GLP-1 receptor agonists is yet unknown. Although meta-analyses of phase-II and phase-III trials studying GLP-1 receptor agonists observe no increase in cardiovascular risk (27), the safety of these agents is currently evaluated in large-sized placebo-controlled cardiovascular outcome trials in patients with type 2 diabetes at high cardiovascular risk. To date, only the results of the ELIXA (28) and LEADER trials (29) have been reported. ELIXA reported neutral effect on cardiovascular events and mortality after 25-month treatment with the GLP-1 receptor agonist lixisenatide (28). However, whether the results of this GLP-1 receptor agonist with a very short half-life, and consequently little effect on RHR, may be extrapolated to all GLP-1 receptor agonists is not clear. Recently, 3.8-year liraglutide treatment in 9340 patients in LEADER was reported to result in a significant reduction in cardiovascular events and mortality, despite an RHR increase of 3 BPM. Notably, all patients in this trial had poorly controlled diabetes and high cardiovascular risk, which precludes generalisation to populations such as those of the current study. Moreover, one could question whether these and upcoming cardiovascular safety trials are of sufficient duration to truly assess the long-term effects of a modest chronic RHR acceleration, as most studies have a follow-up of only several years.

The current study has some limitations. First, we used non-invasive pulse pressure measurement devices to calculate systemic haemodynamic parameters. However, these are well validated against intra-arterial measurements (30). Second, for the acute intervention study, we administered exenatide intravenously, whereas the subcutaneous route is used in clinical practice. Differences in administration route has no effect on time...
to reach therapeutic plasma exenatide levels, whereas it allowed us to perform a blinded study with steady-state exenatide levels (15). However, whether intravenous administration yields similar haemodynamic effects compared with subcutaneous administration remains unstudied, although RHR acceleration is seen with both routes of administration (3, 10). Third, for the prolonged intervention study, we performed single measurements for SNS activity, although it was recently demonstrated that effects of liraglutide may vary throughout the day (5). Fourth, augmentation index is an indirect marker of arterial stiffness. Fifth, although our study was adequately powered to assess the primary endpoint, it may not have been sufficient for secondary endpoints. Finally, effects on glucose and insulin may have contributed to the effects of the GLP-1 receptor agonists (31, 32). However, RHR acceleration with GLP-1 peptide occurs independent of glucose reductions; for example, when glucose and insulin were stabilised during clamped hyperglycaemia (33) or when glucose levels increased during intraduodenal glucose infusion (34).

To conclude, the RHR acceleration that is seen with acute and prolonged GLP-1 receptor agonist administration in overweight type 2 diabetes patients are not explained by changes in SNS activity. Moreover, although with limited statistical power, our data argue against reflex tachycardia caused by vasodilation. In line with animal studies, our results suggest direct sino-atrial stimulation as a probable cause. Whether RHR acceleration during prolonged GLP-1 receptor agonist treatment is truly caused by sino-atrial stimulation and whether it is associated with adverse events needs further study.

Supplementary data
This is linked to the online version of the paper at http://dx.doi.org/10.1530/EJE-16-0507.

Declaration of interest
Through M H Kramer and M Diamant, the VU University Medical Center received research grants from AstraZeneca, Boehringer Ingelheim, Novo Nordisk and Sanofi. All other authors declare that there is no duality of interest associated with their contribution to this manuscript.

Funding
The research leading to these results has received funding from the European Community’s Seventh Framework Programme (FP7/2007–2013) under grant agreement nº 282521 – the SAFEGUARD project. Novo Nordisk A/S kindly provided liraglutide and liraglutide-placebo. Funders had no role in the study design, in collection, analysis and interpretation of data and in the writing of the report or in the decision to submit the article for publication.

Author contribution statement
M M Smits developed the study protocol, obtained the measurements and performed the analyses and wrote the manuscript. L Tonneijck performed measurements and contributed to the discussion and manuscript writing. M Diamant developed the study protocol and was involved in the discussion. T Hoekstra supervised statistical analyses and contributed to editing of the manuscript. M H A Muskiet, M H H Kramer and D H van Raalte contributed to the discussion and edited the manuscript. All authors have approved the final version of this manuscript. All authors had full access to all of the data and can take responsibility for the integrity of the data and the accuracy of the data analysis.

Acknowledgements
The authors are grateful to the study participants whose time and effort was critical to the success of our research protocol. Furthermore, the authors would like to thank Jeannette Boerop for her invaluable help during the test visits. In memory of Prof Michaela Diamant whose experience and expertise were crucial for the design of this study.

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Accepted 24 October 2016

Received 13 June 2016

Revised version received 4 October 2016

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