Advanced vascular imaging

de Boer, Stefanie Amarens

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2017

Link to publication in University of Groningen/UMCG research database


Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
Chapter 6

Effect of linagliptin on pulse wave velocity in early type 2 diabetes: A randomized, double-blind, controlled 26-week trial (RELEASE)


Diabetes Obesity and Metabolism. 2017;19:1147-1154.
ABSTRACT

Aims: To evaluate the effects of the dipeptidyl peptidase-4 (DPP-4) inhibitor linagliptin on aortic pulse wave velocity (PWV) as a surrogate marker of arterial stiffness and early atherosclerosis in people with early type 2 diabetes.

Methods: A total of 45 people with type 2 diabetes (median [interquartile range] age 63 [54-66] years, 61% men, mean ± standard deviation glycated haemoglobin [HbA1c] 6.3±0.4% [45±4.6 mmol/mol]), without cardiovascular disease and naïve to antidiabetic treatment, were randomized (1:1) to treatment with linagliptin 5 mg once daily or placebo for 26 weeks in a double-blind fashion. PWV was assessed at baseline, 4 and 26 weeks of treatment, and again at 30, 4 weeks after treatment. The primary endpoint was between-group difference in PWV (corrected for systolic blood pressure [SBP]) at week 26. Secondary endpoints included differences in central SBP and augmentation index (AIx).

Results: Compared with placebo, 26 weeks of linagliptin decreased PWV by an average of 0.91 m/s (95% confidence interval -1.76 to -0.06; P=.035]. PWV returned to baseline after 4 weeks washout. Differences in central SBP and AIx were not different between linagliptin and placebo. Linagliptin decreased HbA1c (-0.4%; P<.001), fasting plasma glucose (-0.7 mmol/L; P=.002) and triglycerides (-0.49 mmol/L; P=.019) as compared with placebo. The changes in body weight, cholesterol and high-sensitivity C-reactive protein did not differ between groups.

Conclusions: Linagliptin decreased aortic PWV in people with early-stage type 2 diabetes as compared with placebo after 26 weeks of treatment. These results suggest that linagliptin has a favourable effect on arterial stiffness.
INTRODUCTION

People with type 2 diabetes and prediabetes are at increased risk of developing cardiovascular (CV) disease.\(^1\,\,2\) Although modern medicine has provided effective therapy to lower blood glucose levels, people with type 2 diabetes still have a severely increased risk of CV disease.\(^3\) New drugs to treat diabetes should not only lower blood glucose, but also offer CV protection.

Dipeptidyl peptidase (DPP)-4 inhibitors constitute a relatively new class of oral antidiabetic agents. DPP-4 inhibitors inhibit the DPP-4 enzyme, which cleaves incretins, such as glucagon-like peptide 1 (GLP-1), which in turn controls glucose-dependent insulin secretion. DPP-4 inhibitors have been shown to be effective in reducing glycated haemoglobin (HbA\(_1c\)) without inducing hypoglycaemia and have a neutral effect on weight.\(^4\) The CV safety (non-inferiority compared with placebo) of DPP-4 inhibitors (ie, alogliptin, saxagliptin, sitagliptin) has been demonstrated in three published CV outcomes trials (EXAMINE,\(^5\) SAVOR,\(^6\) TECOS\(^7\)) which predominantly included patients with established CV disease.

Furthermore, DPP-4 has several additional effects beyond GLP-1 degradation. DPP-4 is widely distributed in tissues including kidney, intestines, adipose tissue, endothelial cells and bone marrow-derived cells.\(^4\,\,8\) DPP-4 has been shown to cleave multiple substrates, many of which influence the CV system.\(^4\,\,8\) One example substrate is the chemokine stromal cell-derived factor-1α (SDF-1α), which is responsible for the recruitment of endothelial progenitor cells.\(^8\) Moreover, DPP-4 is expressed on blood T cells and is associated with the immune system.\(^4\,\,9\) Consequently, DPP-4 enzyme inhibition might result in favourable CV effects beyond glucose-lowering. Indeed, animal studies, as well as some clinical studies, have shown favourable CV effects, such as reduction of silent inflammation and oxidative stress, lower cholesterol levels and improvement of endothelial function.\(^4\,\,10\,\,12\) Nevertheless, DPP-4 enzyme inhibition might also result in unfavourable CV effects; an increased rate of hospitalization for heart failure was observed in one CV outcome trial.\(^6\)

Arterial stiffness can be non-invasively assessed as aortic pulse wave velocity (PWV) and by pulse wave analysis as central systolic blood pressure (SBP) and augmentation index (Alx). PWV is an integrated index of arterial function and structure and hence a marker of early atherosclerosis.\(^13\) A higher PWV is associated with a more stiffened artery and an increased risk of CV events.\(^14\) Overall, PWV is a strong independent predictor of future CV events and all-cause mortality in the general population. Furthermore, PWV is also a relevant predictor in populations with high CV risk such as those with type 2 diabetes, hypertension and kidney disease.\(^13\,\,15\) Whether DPP-4 inhibitors such as linagliptin attenuate arterial stiffness has not, however, been studied previously in a double-blind randomized placebo-controlled clinical trial.
We hypothesized that treatment with linagliptin, when started early in the course of type 2 diabetes, would result in favourable vascular effects. The primary objective of the present study, therefore, was to evaluate the effects of 26 weeks of linagliptin treatment on PWV, and secondly, on central SBP and Alx in people with early-stage type 2 diabetes.

METHODS

The RELEASE study was a single centre, randomized, prospective, double-blind, placebo-controlled, parallel-group phase III study. The study started in February 2014 and was clinically completed in March 2016. The protocol was reviewed and approved by the Medical Ethical Institutional Review Board of the University Medical Center Groningen (UMCG). The study was carried out according to the principles of the Declaration of Helsinki and according to Good Clinical Practice guidelines. All participants gave written informed consent before entering the study. The trial was registered with clinicaltrials.gov (NCT02015299).

Study design and population

Potentially eligible participants were selected from the outpatient clinic of the Vascular Medicine Department of the UMCG, and were recruited by advertisement in a local newspaper and from several general practices. Eligibility criteria are described in detail in Supplemental File 1. Briefly, participants were men and women aged ≥30 and ≤70 years, diagnosed with type 2 diabetes according to American Diabetes Association criteria, and were required to have an assessable PWV at screening and to be on a stable dose of blood pressure- and/or lipid-lowering medication. Exclusion criteria were: current use of glucose-lowering drugs, diagnosis of CV disease (defined as stable coronary artery disease or history of an acute coronary syndrome, stroke or transient ischaemic attack, or peripheral artery disease); and uncontrolled hypertension (SBP>160 mm Hg or diastolic blood pressure >100 mm Hg).

All eligible participants underwent a screening visit, during which the feasibility of PWV assessment was performed. The inclusion visit took place within 8 weeks after the screening visit. Follow-up visits were scheduled at weeks 4, 8, 16 and 26 (the completion visit), and there was a 4-week post-treatment (washout) follow-up at week 30. PWV, central SBP and Alx were assessed at baseline, 4 and 26 weeks of treatment, and at 30 weeks. The visit at week 8 consisted of a telephone consultation, and at week 16 an interim visit was planned. During every visit, participants were asked to report adverse events.
**Intervention**

Participants were randomized in a 1:1 ratio to receive either linagliptin 5 mg once daily or matching placebo for 26 weeks. To minimize adverse events of active glucose-lowering drugs, especially the risk of hypoglycaemic events, placebo was chosen for the control group. Randomization was performed using minimization software (MinimPy, downloaded at http://minimpy.sourceforge.net).\(^{17}\) Allocation was stratified by age (30-49 vs 50-70 years), concomitant use of drugs that intervene in the renin-angiotensin-aldosterone system (angiotensin receptor blockers and angiotensin-converting enzyme inhibitors) and smoking status (current smoking vs non-smoking). Medication bottles were number-coded to blind both participants and investigators. Participants were requested to return the medication bottles at every visit to calculate drug compliance. All participants in the trial received lifestyle advice in accordance with the Dutch General Practitioner standards.

Rescue therapy was initiated if any of the following criteria were met: the participant had a fasting plasma glucose level >15 mmol/L or HbA\(_{1c}\) >8.0% (64 mmol/mol). Preferably, a sulphonylurea derivate was started because these agents have not demonstrated an influence on PWV.\(^{18}\)

**Clinical and laboratory assessments**

Height, weight and waist circumference were measured, and body mass index (kg/m\(^2\)) was calculated. All blood samples were obtained in the morning after at least 8 hours of overnight fasting for measuring plasma glucose, HbA\(_{1c}\), full blood cell count, lipid profile, high-sensitivity C-reactive protein (hsCRP), liver enzymes and serum creatinine. Measurements were not performed after fasting in the interim visit at week 16. All measurements were performed in the national accredited clinical laboratory unit of the UMCG, according to standard procedures.

**Arterial stiffness**

All vascular measurements were performed at our vascular laboratory and are described elsewhere in detail.\(^{19}\) Briefly, pressure waves were recorded sequentially in the left carotid and femoral arteries with the use of the Sphygmocor EM-3 device (AtCor Medical, West Ryde, Australia, software version 8.2). The PWV was calculated by dividing travelled distance by transit time (\(\text{PWV} = \frac{\text{distance [meters]}}{\text{transit time [seconds]}}\)).\(^{20}\) Pulse wave analysis was performed with the same system to estimate the aortic pressure. Aortic pressure waveform was estimated from the radial artery using the transfer function\(^{21}\) and central SBP and AIx were obtained. The AIx is defined as the second peak minus the first peak of the central arterial waveform, expressed as a percentage of the pulse pressure, and standardized to a heart rate of 75 beats per minute.
Endpoints
The primary endpoint was between group difference in PWV (corrected for SBP) at week 26. In addition to the primary endpoint, the difference in PWV at week 4 and week 30 (washout) was also assessed. Secondary endpoints were the difference in central SBP and Alx over time. Other outcome endpoints were differences from baseline in clinical and laboratory assessments.

Safety endpoints included the incidence and intensity of adverse events (AEs), including AEs of special interest, and changes in vital signs and laboratory tests if they were clinically relevant. AEs of special interest were: hepatic events; hypersensitivity reactions; pancreatitis; renal events; and skin lesions.

Sample size determination
A sample size of 36 participants completing the 26 weeks treatment provided 80% power at a 2-sided α of 5% to detect a 1.0 m/s reduction in PWV, assuming a standard deviation of 1.04 based on previous studies. In order to have at least 36 participants completing the primary endpoint, we aimed to include 20 participants in each treatment group. When participants discontinued after the randomization, those participants could be substituted according to protocol.

Statistical methods
Data from all included participants were used in the analysis and missing values were not imputed. Data collected after rescue medication were not used for efficacy analysis. Means from normally distributed variables were compared using an independent-sample Student t-test or Mann–Whitney U-test when appropriate. Discrete variables were compared using the chi-squared or Fisher’s exact test, as appropriate.

The primary endpoint (PWV between-group difference at week 26) was evaluated using the generalized estimating equations (GEE) approach with an unstructured covariance matrix on an intention-to-treat basis, resulting in all participants with at least 1 measurement being included. PWV was entered as the dependent variable in the model. Treatment group and visit were added in the model as factors, and SBP as a covariate. An interaction between visit number and treatment group was also added into the model in a second step. From baseline to week 26 the model included the PWV measurements obtained at baseline, 4 and 26 weeks. From baseline to week 30 the GEE included PWV measurements obtained at baseline and 30 weeks. The estimated marginal means for week 4, 26 and 30 within a group compared with their baseline measurements and the difference between groups at each visit were evaluated and compared with appropriate correction for pairwise comparisons. Treatment effects on central SBP and Alx were analysed similarly to PWV, but without SBP as a covariate in the model. Between-group differences from baseline to 26 weeks for clinical outcomes were analysed using the calculated deltas (Δ).
To explore whether associations between change in PWV, central SBP, Alx and HbA$_1c$ were correlated, a Pearson or Spearman correlation coefficient ($r$) was calculated when appropriate on the calculated $\Delta$ from baseline to 26 weeks.

All analyses were performed using SPSS (released 2013; IBM SPSS Statistics for Windows, Version 22.0, IBM Corp, Armonk, New York). $P$ values <.05 were taken to indicate statistical significance.

**RESULTS**

**Study population**

Of the 50 participants screened, 45 were randomized and 44 started medication. Six participants were not eligible: in 4 participants PWV measurement was unreliable as a result of arrhythmia and 2 participants had to be withdrawn because of current health concerns (Figure 1); therefore, 44 participants (27 men and 17 women) were randomly assigned to treatment with linagliptin or placebo. Participants had a median (interquartile range [IQR]) age of 63 (54-66) years and the median diabetes (IQR) duration was 1 (0-3.5) year. Baseline characteristics were well balanced between the groups (Table 1).

---

**Screened** ($n=50$)

- Randomized ($n=45$)
  - Placebo ($n=22$)
    - Not eligible ($n=5$)
      - No reliably assessable PWV (4)
      - Health concern (1)
    - Excluded ($n=1$)
      - Health concern (1)
  - Linagliptin 5 mg ($n=22$)
    - Discontinued ($n=1$)
      - Non-compliance (1)
    - Full analysis primary outcome ($n=19$)
      - Full analysis secondary outcomes ($n=21$)
  - Protocol violation ($n=2$)
    - Start antihyperglycemic rescue therapy (SU derivate) and metformin (1)
    - Start statin (1)
    - Discontinued ($n=1$)
      - Withdrew consent (1)
    - Full analysis primary outcome ($n=19$)
      - Full analysis secondary outcomes ($n=19$)

**Figure 1 | Disposition of study participants.**

SU=sulphonylurea
### Table 1 | Demographic and baseline disease characteristics of enrolled participants.

<table>
<thead>
<tr>
<th></th>
<th>Linagliptin (n=22)</th>
<th>Placebo (n=22)</th>
<th>Total (n=44)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men, n (%)</strong></td>
<td>13 (59)</td>
<td>14 (64)</td>
<td>27 (61)</td>
</tr>
<tr>
<td><strong>Median (IQR) age, years</strong></td>
<td>63 (52-66)</td>
<td>62 (56-69)</td>
<td>63 (54-66)</td>
</tr>
<tr>
<td><strong>White ethnicity, n (%)</strong></td>
<td>18 (82)</td>
<td>22 (100)</td>
<td>40 (91)</td>
</tr>
<tr>
<td><strong>Current smoker, n (%)</strong></td>
<td>5 (23)</td>
<td>2 (9)</td>
<td>7 (16)</td>
</tr>
<tr>
<td><strong>Median (IQR) diabetes duration, years</strong></td>
<td>1.5 (0-5)</td>
<td>1.0 (0-3.3)</td>
<td>1.0 (0.0-3.5)</td>
</tr>
<tr>
<td><strong>Median (IQR) body mass index, kg/m²</strong></td>
<td>32.3(27.8-38.2)</td>
<td>29.0 (27.4-34.2)*</td>
<td>30.4 (27.5-35.8)</td>
</tr>
<tr>
<td><strong>Weight, kg</strong></td>
<td>97.9±17.6</td>
<td>95.3±13.2</td>
<td>96.6±15.4</td>
</tr>
<tr>
<td><strong>Fasting plasma glucose, mmol/L</strong></td>
<td>7.5±0.9</td>
<td>7.4±1.0*</td>
<td>7.4 ±1.0</td>
</tr>
<tr>
<td><strong>HbA1c, %</strong></td>
<td>6.3±0.4</td>
<td>6.2±0.5</td>
<td>6.3 ±0.4</td>
</tr>
<tr>
<td><strong>HbA1c, mmol/mol</strong></td>
<td>45±4.2</td>
<td>45±5.0</td>
<td>45±4.6</td>
</tr>
<tr>
<td><strong>Total cholesterol, mmol/L</strong></td>
<td>4.8±1.1</td>
<td>4.7±0.7</td>
<td>4.7±0.95</td>
</tr>
<tr>
<td><strong>HDL cholesterol, mmol/L</strong></td>
<td>1.4±0.3</td>
<td>1.4±0.4</td>
<td>1.37±0.32</td>
</tr>
<tr>
<td><strong>LDL cholesterol, mmol/L</strong></td>
<td>3.2±1.2</td>
<td>2.9±0.9</td>
<td>3.09±1.02</td>
</tr>
<tr>
<td><strong>Triglycerides, mmol/L</strong></td>
<td>1.48±0.6</td>
<td>1.69±1.0</td>
<td>1.59±0.83</td>
</tr>
<tr>
<td><strong>Median (IQR) hsCRP, mg/L</strong></td>
<td>1.7 (0.8-3.0)</td>
<td>1.1 (0.6-3.5)</td>
<td>1.15 (0.70-3.08)</td>
</tr>
<tr>
<td><strong>eGFR, ml/min/1.73m²</strong></td>
<td>88±12</td>
<td>81±16</td>
<td>85±14 (78-94)</td>
</tr>
<tr>
<td><strong>SBP, mm Hg</strong></td>
<td>139±14</td>
<td>139±13</td>
<td>139±14</td>
</tr>
<tr>
<td><strong>DBP, mmHg</strong></td>
<td>88±10</td>
<td>88±9</td>
<td>88±10</td>
</tr>
<tr>
<td><strong>Medication, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statin</td>
<td>11 (50%)</td>
<td>13 (59%)</td>
<td>24 (54.5%)</td>
</tr>
<tr>
<td>Antihypertensive</td>
<td>10 (46%)</td>
<td>12 (55%)</td>
<td>22 (50%)</td>
</tr>
<tr>
<td><strong>Arterial stiffness</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central SBP, mm Hg</td>
<td>134±15</td>
<td>134±14</td>
<td>134±14</td>
</tr>
<tr>
<td>Median (IQR) Alx, %</td>
<td>21 (17-25)</td>
<td>20 (15-28)</td>
<td>21 (16-26)</td>
</tr>
<tr>
<td>PWV, m/s</td>
<td>8.7±1.6</td>
<td>8.8±1.2</td>
<td>8.7±1.4</td>
</tr>
</tbody>
</table>

Data are mean ± standard deviation, unless otherwise indicated. *n=21

Abbreviations: Alx=augmentation index; DBP=diastolic blood pressure; eGFR=estimated glomerular filtration rate; PWV=pulse wave velocity; SBP=systolic blood pressure.

After start of the medication, 2 participants discontinued treatment, 1 through non-compliance [<80%] and 1 participant withdrew consent. Additionally, 2 participants were withdrawn, 1 as a consequence of starting statin therapy, and 1 started a sulphonylurea derivat (rescue therapy, between visits 2 and 3) and metformin (between visits 3 and 4) to control hyperglycaemia (Figure 1). Overall study medication compliance was 99%. A total of 21 participants were available for analysis in the linagliptin group and 19 in the placebo group. Because PWV measurements of 2 participants at week 26 were not successfully obtained, the primary endpoint analysis considered 19 participants in the linagliptin and 19 in the placebo group.
Primary endpoint
After 26 weeks of therapy, PWV was significantly lower in the linagliptin group than the placebo group, with a between-group difference of 0.91 m/s (95% confidence interval [CI] 0.06-1.76; \(P=0.035\)), as shown in Figure 2. In addition, PWV changed from 8.7 m/s (95% CI 8.0-9.3) at baseline, to 8.3 m/s (95% CI 7.7-8.8) at 4 weeks, to 8.3 m/s (95% CI 7.8-8.9) at 26 weeks in the linagliptin group and from 8.8 m/s (95% CI 8.3-9.3), to 8.8 m/s (95% CI 8.1-9.4), to 9.2 m/s (95% CI 8.6-9.8) in the placebo group. After 4 weeks washout at 30 weeks, PWV in both groups returned to baseline (linagliptin 8.8 m/s [95% CI 8.0-9.7], placebo 8.9 m/s [95% CI 8.2-9.6]; Supplemental Table 1).

Secondary endpoints
As shown in Table 2, central SBP and Alx did not differ significantly from baseline between the linagliptin and placebo groups throughout the treatment (at 26 weeks: \(\Delta\text{SBP}=-2.8\) [95% CI -15.8 to 10.2], \(P=0.674\) and \(\Delta\text{Alx}=-0.7\) [95% CI -4.0 to 3.5], \(P=0.738\)), as well as at week 30 (after washout).

Table 2 | Changes in secondary endpoints induced by linagliptin or placebo at different time points.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time</th>
<th>Linagliptin group</th>
<th>Placebo group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n) Mean (95% CI)</td>
<td>(n) Mean (95% CI)</td>
<td></td>
</tr>
<tr>
<td>Central SBP, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>21 134 (128-140)</td>
<td>21 134 (128-139)</td>
<td></td>
</tr>
<tr>
<td>4 weeks</td>
<td>20 131 (124-138)</td>
<td>20 134 (128-139)</td>
<td></td>
</tr>
<tr>
<td>26 weeks</td>
<td>19 134 (127-141)</td>
<td>17 137 (126-148)</td>
<td></td>
</tr>
<tr>
<td>Washout</td>
<td>20 132 (126-139)</td>
<td>17 136 (129-143)</td>
<td></td>
</tr>
<tr>
<td>Alx, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>21 19.7 (16.1-23.3)</td>
<td>21 21.5 (18.2-25.6)</td>
<td></td>
</tr>
<tr>
<td>4 weeks</td>
<td>20 19.8 (16.6-23.1)</td>
<td>20 20.9 (17.2-24.7)</td>
<td></td>
</tr>
<tr>
<td>26 weeks</td>
<td>19 20.6 (17.6-23.5)</td>
<td>17 21.3 (18.3-24.3)</td>
<td></td>
</tr>
<tr>
<td>Washout</td>
<td>20 20.6 (17.4-23.8)</td>
<td>17 22.7 (19.5-25.9)</td>
<td></td>
</tr>
</tbody>
</table>

Data presented as estimated marginal means and 95% CI.
Abbreviations: Alx=augmentation index; SBP=systolic blood pressure

In the linagliptin group, a positive correlation was observed between the decrease in PWV from baseline to week 26 and central SBP (\(R=0.53, P=0.024\)). Conversely, no correlation was found between the before-mentioned decrease in PWV and the Alx (\(R=-0.15, P=0.565\)). Interestingly, PWV change from baseline to week 26 tended to correlate with change in HbA\(_1c\) (\(R=0.42, P=0.071\)) but not with change in glucose (\(R=-0.104, P=0.672\)) or triglycerides (\(R=-0.113, P=0.644\)). In the placebo group, no significant correlations were observed between change in PWV and central SBP, Alx, HbA\(_1c\), glucose or triglycerides.
**Complementary clinical and laboratory endpoints**

At 26 weeks of therapy, linagliptin decreased HbA\textsubscript{1c} (-0.4%; \(P<0.001\)), fasting plasma glucose (-0.7 mmol/L; \(P=0.002\)) and triglyceride levels (-0.49 mmol/L; \(P=0.019\)) as compared with placebo. Changes in body weight, cholesterol and hsCRP did not differ between groups throughout the study. The details of the changes in clinical and laboratory variables induced by treatment with linagliptin or placebo at different study time points are shown in Table 3.

**Table 3** | Changes in clinical and laboratory parameters induced by linagliptin or placebo at different time points.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time</th>
<th>Linagliptin group</th>
<th>Placebo group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time</td>
<td>(n)</td>
<td>Mean (SD) or Median</td>
</tr>
<tr>
<td>HbA\textsubscript{1c}, %</td>
<td>Baseline</td>
<td>22</td>
<td>6.3±0.4</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td>21</td>
<td>6.1±0.4</td>
</tr>
<tr>
<td></td>
<td>26 weeks</td>
<td>21</td>
<td>6.1±0.3</td>
</tr>
<tr>
<td></td>
<td>Washout</td>
<td>21</td>
<td>6.0±0.3</td>
</tr>
<tr>
<td>HbA\textsubscript{1c}, mmol/mol</td>
<td>Baseline</td>
<td>21</td>
<td>6.0±0.3</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td>22</td>
<td>45±4.2</td>
</tr>
<tr>
<td></td>
<td>26 weeks</td>
<td>21</td>
<td>43±4.3</td>
</tr>
<tr>
<td></td>
<td>Washout</td>
<td>21</td>
<td>43±3.4</td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/L</td>
<td>Baseline</td>
<td>21</td>
<td>42±3.3</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td>21</td>
<td>42±3.8</td>
</tr>
<tr>
<td></td>
<td>26 weeks</td>
<td>22</td>
<td>7.5±0.9</td>
</tr>
<tr>
<td></td>
<td>Washout</td>
<td>21</td>
<td>7.1±0.8</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>Baseline</td>
<td>21</td>
<td>7.1±0.6</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td>21</td>
<td>7.5±0.7</td>
</tr>
<tr>
<td></td>
<td>26 weeks</td>
<td>22</td>
<td>97.9±17.6</td>
</tr>
<tr>
<td></td>
<td>Washout</td>
<td>21</td>
<td>97.8±17.7</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>Baseline</td>
<td>21</td>
<td>97.9±17.9</td>
</tr>
<tr>
<td></td>
<td>26 weeks</td>
<td>21</td>
<td>97.8±18.0</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>Baseline</td>
<td>22</td>
<td>1.3±0.3</td>
</tr>
<tr>
<td></td>
<td>26 weeks</td>
<td>21</td>
<td>1.4±0.3</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>Baseline</td>
<td>22</td>
<td>3.2±1.2</td>
</tr>
<tr>
<td></td>
<td>26 weeks</td>
<td>21</td>
<td>3.1±1.1</td>
</tr>
<tr>
<td>hsCRP, mg/L</td>
<td>Baseline</td>
<td>22</td>
<td>1.5±0.6</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td>21</td>
<td>1.3±0.5</td>
</tr>
<tr>
<td></td>
<td>26 weeks</td>
<td>22</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>Washout</td>
<td>21</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Abbreviation: SD=standard deviation
Safety
Two serious AEs were reported, both with an unlikely causal relationship between the event and the study medication: 1 participant required hospitalization for a kidney contusion after trauma (linagliptin group) and 1 participant required hospitalization for an unprovoked pulmonary embolism (placebo group). One AE of special interest occurred in the linagliptin group: alanine aminotransferase increased to 165 U/L after 6 weeks of study medication, decreased to 18 U/L after 10 weeks, and remained normal during follow-up.

DISCUSSION
In this randomized placebo-controlled trial we showed that 26 weeks of treatment with the DPP-4 inhibitor linagliptin decreased PWV, a marker of arterial stiffness, in participants with type 2 diabetes naïve to antidiabetic treatment. These results support earlier observations from (pre)clinical studies which have suggested that DPP-4 inhibitors may exert favourable vascular effects beyond glucose-lowering.4

Compared with placebo, linagliptin decreased PWV significantly after 26 weeks of treatment by 0.91 m/s indicating CV risk reduction.14 An increase of 1.0 m/s has been associated with an age-, sex- and risk factor-adjusted risk increase of ~15% in CV events, CV mortality and all-cause mortality.14,15,25 A literature search also revealed other studies that have assessed the effect of DPP-4 inhibitors on PWV. One study included 51 well-regulated participants with type 2 diabetes and found a change in PWV of -0.4 m/s after 12 weeks of treatment with vildagliptin or sitagliptin.26 Another study in 32 drug-naïve participants with type 2 diabetes found a change of -0.3 m/s after 26 weeks’ treatment with metformin and
vildagliptin. A cross-over study with saxagliptin and placebo in 42 participants showed a change of -0.21 m/s after treatment with saxagliptin compared with placebo. Although in these previous studies a trend in PWV reduction was reported, the change in PWV did not reach statistical significance. In contrast to the present study, 2 of the previous studies did not include a placebo arm. Furthermore, none of the other studies were primarily powered to determine an effect on PWV, which makes the results subject to bias.

We observed that the PWV results already decreased after 4 weeks of linagliptin and returned to baseline 4 weeks after stopping treatment. This indicates that the decrease in PWV is a fast-acting and reversible dynamic effect. Despite the effect on PWV, linagliptin did not change central SBP or AIx over time. The lack of effect on central SBP is consistent with other studies. AIx is determined by the degree of peripheral wave reflection and is considered an indirect surrogate measure of arterial stiffness. Compared with PWV, AIx is influenced by heart rate and blood pressure to a greater extent than PWV and is a less reliable marker of arterial stiffness. Hence, the lack of effect on AIx should not be interpreted as if linagliptin does not ameliorate arterial stiffness. Currently, PWV is the most robust and validated marker of arterial stiffness and is considered the “gold standard” for arterial stiffness, because it is accurate, reproducible and has been shown to predict CV events.

Although, PWV is considered the gold standard, in clinical practice, the measurements are sometimes difficult to obtain. To this end, we screened participants on having an assessable PWV at screening. Yet, despite these precautionary measurements, PWV measurements failed in 2 participants at week 26.

A correlation between change from baseline to week 26 in PWV and central SBP was observed. This was expected, because PWV is influenced by blood pressure; therefore, blood pressure was added in the GEE as a covariate to correct for SBP. Interestingly, PWV is associated with microvascular dysfunction in both the brain (cerebral small vessel lesions) and kidney (microalbuminuria). As linagliptin decreased PWV but had no effect on central SBP, it may be the case that linagliptin has more peripheral (cq micro) vascular effects independently of systemic blood pressure. Indeed, recent studies have shown that linagliptin improves microvascular function by increasing axon reflex-dependent vasodilation, improving microvascular retinal blood flow, cerebrovascular function and remodeling and possibly albuminuria. Moreover, PWV can be decreased by peripheral vasodilation, which does not affect central aortic pressure.

The underlying mechanism of the decrease in arterial stiffness of linagliptin is not completely clear. DPP-4 inhibitors inhibit the enzyme DPP-4, which cleaves multiple peptides (also known as catalytic function). Many of those peptides influence the CV system, as reviewed elsewhere. As DPP-4 enzyme cleaves the incretin hormone GLP-1, which controls glucose-dependent insulin secretion, improved glycaemic control could be an underlying mechanism. Improved glycaemic control with metformin has also been shown to decrease PWV in a previous study. Other oral antidiabetes drugs, such as pioglitazone
Effect of linagliptin on arterial stiffness

and empagliflozin,\textsuperscript{38} also decrease PWV, whereas glibenclamide and voglibose have been shown not to affect PWV.\textsuperscript{18} Another clinical study in participants with uncomplicated type 2 diabetes recently showed that exenatide (a GLP-1 agonist) compared with placebo decreased PWV.\textsuperscript{39} As GLP-1 has also non-metabolic effects that may lead to beneficial effects on the CV system, the underlying mechanism of decreased PWV is incompletely understood.\textsuperscript{8} Another mechanism of decreased PWV by DPP-4 inhibitors could be attributable to an increase in other peptides cleaved by DPP-4, such as SDF-1\textsubscript{\alpha} or Substance P.\textsuperscript{4} SDF-1\textsubscript{\alpha} upregulation is associated with increased circulating endothelial progenitor cells,\textsuperscript{10} which have been associated with lower arterial stiffness in participants with type 2 diabetes;\textsuperscript{4} however, DPP-4 itself also has a non-catalytic function which might influence the CV system.

Recently published, long-term, large prospective, randomized, double-blind trials with DPP-4 inhibitors have demonstrated no clear CV benefit of adding a DPP-4 inhibitor to usual care\textsuperscript{5-7}; however, these trials were designed to demonstrate CV safety and their external validity is low, considering the study population in the present study. The results of two large trials on CV safety of linagliptin ([CAROLINA\textsuperscript{41}, CARMELINA\textsuperscript{42}]) are pending. In these trials, DPP-4 inhibitors are studied on top of usual care in patients at substantial CV risk. These trials will provide more definitive evidence on the long-term CV effects of linagliptin and whether potential CV protective effects are dependent on glycaemic control.

The present study has some limitations. First, the duration of treatment was 6 months, so whether long-term changes would be sustained is unknown. Second, this was a relatively small study with a limited number of participants involving surrogate endpoints, and therefore, no conclusion regarding CV outcomes can be drawn from these results. Nevertheless, the study provides more insight into the effect of DPP-4 inhibitors on vascular function. Third, the results were obtained in relatively healthy people with diabetes so the findings might not be extrapolated to people with type 2 diabetes with a longer diabetes duration or with advanced atherosclerotic disease. Fourth, the trial lacked an active control group so that the glucose-independent effects of linagliptin on PWV could not be excluded definitively.

In conclusion, compared with placebo, linagliptin decreases aortic PWV as a measure of arterial stiffness and predictor of CV events, in participants with early-stage type 2 diabetes after 26 weeks of treatment. Further studies are needed to assess whether a reduction in aortic PWV is sustained in the long term and translates into an improvement in CV outcome.

Acknowledgments

The authors wish to thank the study participants, the general practices, as well as M. G. Piersma-Wichers and H. L. Lutgers from CERTE Groningen. We also thank A. I. van Gessel and S. C. van Zande (vascular technicians, UMCG, the Netherlands), I. T. Wilts (MD, researcher, UMCG, the Netherlands) and B. T. Fokkens (researcher, UMCG, the Netherlands) for their important contributions.
**Funding Information**
This study was supported by Boehringer Ingelheim (Alkmaar, the Netherlands). Boehringer Ingelheim was not involved in the design of the study, collection, management, analysis, and interpretation of the data, writing of the report, or the decision to submit the paper for publication.

**Conflict of interest**
The authors have no conflict of interest to declare.

**Author contributions**
S. A. d. B. collected, analysed and interpreted the data, and wrote the manuscript. H. J. L. H analysed and interpreted the data, conceived and designed the study and reviewed the manuscript critically for intellectual content. L. E. J. O. analysed and interpreted the data and reviewed the manuscript critically for intellectual content. A. M.v. R. collected and analysed the data, and reviewed the manuscript critically for intellectual content. P. W. K conceived and designed the study and reviewed the manuscript critically for intellectual content. A. J. S. analysed and interpreted the data and reviewed the manuscript critically for intellectual content. R. H. J. A. S. analysed the data, conceived and designed the study and reviewed the manuscript critically for intellectual content. J. D. L. conceived and designed the study and reviewed the manuscript critically for intellectual content, D. J. M. collected, analysed and interpreted the data, conceived and designed the study and reviewed the manuscript critically for intellectual content. S. A. d. B and D. J. M. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors approved the final version. Parts of this study were presented at the American Heart Association, November 12 to 16, 2016, New Orleans.
REFERENCES


Supplemental File 1 | IN AND EXCLUSION CRITERIA

Study Population

Population (base)
We anticipate to include a total of 40 persons with diabetes, with 20 subjects per treatment arm

Inclusion criteria
- Men and women, age 30 to 70 years, AND
- Treatment naïve type 2 diabetes, as defined as documentation of one of the following (American Diabetes Association definition):
  - Fasting plasma glucose ≥7.0 mmol/l, OR
  - Random plasma glucose ≥11.1 mmol/l, OR
  - HbA1c ≥6.5%
- Written informed consent
- Assessable Pulse Wave Velocity measurement at screening
- Be on a stable dose of blood pressure and/or lipid lowering medication for more than 4 weeks.

Exclusion criteria
- Current or previous use of glycemic control medications, defined as used for a minimal period of 30 consecutive days and within one year prior to inclusion
- Type 1 diabetes
- Gestational diabetes mellitus
- Other specific types of diabetes due to other causes, e.g., genetic defects in β-cell function, genetic defects in insulin action, diseases of the exocrine pancreas (such as cystic fibrosis), and drug- or chemical-induced (such as in the treatment of HIV/AIDS or after organ transplantation)
- Uncontrolled hypertension, defined as persisting systolic blood pressure >160 or a diastolic blood pressure >100 mmHg without evidence of white coat hypertension.
- Severe dyslipidemia indicating primary dyslipidemia, defined as total cholesterol >8 mmol/l, tryglicerides >10 mmol/l of high density lipoprotein cholesterol <0.6 mmol/l
- Current use of weight loss medication or previous weight loss surgery
- History of severe gastrointestinal disease
- Clinical contraindications to DPP4-inhibitors
- Previous cardiovascular disease, defined as stable coronary artery disease or acute coronary syndrome, stroke or transient ischemic attack, peripheral artery disease
- Symptomatic heart failure, New York Heart Association (NYHA) class II-IV
- Women who are currently pregnant, planning to become pregnant, breastfeeding women, or women with child bearing potential not using appropriate contraceptive measures
- Clinically significant liver disease or hepatic function greater than 3 times upper limit of normal
- Known impaired renal function or eGFR < 30 ml/min/1.73m²
- Patients who are mentally incompetent and cannot sign a Patient Informed Consent
- Current active malignancy or in the previous 6 months
- Documented HIV infection
- Use of rifampicin
- Known or suspected allergy to ¹⁸F-FDG or its component
Supplemental Table 1 | Changes in PWV induced by linagliptin versus of placebo at different time point.

<table>
<thead>
<tr>
<th>Mean</th>
<th>PWV Mean (95%CI)</th>
<th>Change from baseline Within-group difference*</th>
<th>Between-group difference†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (95% CI)</td>
<td>P Value‡</td>
<td>Mean (95% CI) P Value§</td>
</tr>
<tr>
<td>Baseline</td>
<td>Placebo (n=22)</td>
<td>8.79 (8.31 to 9.26)</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>Linagliptin (n=22)</td>
<td>8.66 (8.01 to 9.32)</td>
<td>-0.09 (-1.01 to 0.814)</td>
</tr>
<tr>
<td>Week 4</td>
<td>Placebo (n=20)</td>
<td>8.76 (8.13 to 9.40)</td>
<td>-0.02 (-0.53 to 0.48)</td>
</tr>
<tr>
<td></td>
<td>Linagliptin (n=19)</td>
<td>8.25 (7.72 to 8.78)</td>
<td>-0.41 (-0.92 to 0.09)</td>
</tr>
<tr>
<td>Week 26</td>
<td>Placebo (n=19)</td>
<td>9.17 (8.57 to 9.77)</td>
<td>0.38 (-0.30 to 1.06)</td>
</tr>
<tr>
<td></td>
<td>Linagliptin (n=19)</td>
<td>8.25 (7.65 to 8.85)</td>
<td>-0.41 (-1.22 to 0.40)</td>
</tr>
<tr>
<td>Week 30</td>
<td>Placebo (n=16)</td>
<td>8.91 (8.18 to 9.64)</td>
<td>0.13 (-0.74 to 0.99)</td>
</tr>
<tr>
<td></td>
<td>Linagliptin (n=20)</td>
<td>8.82 (7.96 to 9.68)</td>
<td>0.16 (-0.75 to 1.06)</td>
</tr>
</tbody>
</table>

*The mean change from baseline within groups were assessed using a generalized linear model including the measurements obtained at baseline, 4 and 26 weeks adjusted for systolic blood pressure. For week 30 (washout) within groups effects were assessed using a generalized linear model including baseline PWV adjusted for systolic blood pressure.
†Between-group effects were assessed using a generalized linear model including the measurements obtained at baseline, 4 and 26 weeks adjusted for systolic blood pressure. For week 30 (washout) between-group effects were assessed using a generalized linear model including baseline PWV and adjusted for systolic blood pressure.
‡The result of a pairwise comparison of the estimated marginal means within a group compared to baseline.
§The result of a pairwise comparison of the estimated marginal means between group by visit.