GLP-1–Based Therapies Have No Microvascular Effects in Type 2 Diabetes Mellitus
An Acute and 12-Week Randomized, Double-Blind, Placebo-Controlled Trial

Mark M. Smits, Lennart Tonneijck, Marcel H.A. Muskiet, Trynke Hoekstra, Mark H.H. Kramer, Michaela Diamant,† Erik H. Serné,* Daniël H. van Raalte*

Objective—To assess the effects of glucagon-like peptide (GLP)-1–based therapies (ie, GLP-1 receptor agonists and dipeptidyl peptidase-4 inhibitors) on microvascular function in patients with type 2 diabetes mellitus.

Approach and Results—We studied 57 patients with type 2 diabetes mellitus (mean±SD age: 62.8±6.9 years; body mass index: 31.8±4.1 kg/m²; HbA₁c [glycated hemoglobin] 7.3±0.6%) in an acute and 12-week randomized, placebo-controlled, double-blind trial conducted at the Diabetes Center of the VU University Medical Center. In the acute study, the GLP-1 receptor agonist exenatide (therapeutic concentrations) or placebo (saline 0.9%) was administered intravenously. During the 12-week study, patients received the GLP-1 receptor agonist liraglutide (1.8 mg daily), the dipeptidyl peptidase-4 inhibitor sitagliptin (100 mg daily), or matching placebos. Capillary perfusion was assessed by nailfold skin capillary videomicroscopy and vasomotion by laser Doppler fluxmetry, in the fasting state and after a high-fat mixed meal. In neither study, treatment affected fasting or postprandial capillary perfusion compared with placebo (P>0.05). In the fasting state, acute exenatide infusion increased neurogenic vasomotion domain power, while reducing myogenic domain power (both P<0.05). After the meal, exenatide increased endothelial domain power (P<0.05). In the 12-week study, no effects on vasomotion were observed.

Conclusions—Despite modest changes in vasomotion, suggestive of sympathetic nervous system activation and improved endothelial function, acute exenatide infusion does not affect skin capillary perfusion in type 2 diabetes mellitus. Twelve-week treatment with liraglutide or sitagliptin has no effect on capillary perfusion or vasomotion in these patients. Our data suggest that the effects of GLP-1–based therapies on glucose are not mediated through microvascular responses. (Arterioscler Thromb Vasc Biol. 2016;36:2125-2132. DOI: 10.1161/ATVBAHA.116.307930.)

Key Words: blood pressure ■ diabetes mellitus ■ DPP-4 inhibitors ■ GLP-1 receptor agonists ■ microcirculation

Pharmacological agents that are based on the gut-derived hormone glucagon-like peptide (GLP)-1 are increasingly used for hyperglycemia management in type 2 diabetes mellitus. Both GLP-1 receptor agonists and inhibitors of the GLP-1–degrading enzyme dipeptidyl peptidase-4 (ie, DPP-4 inhibitors) increase insulin and reduce glucagon secretion, thereby lowering plasma glucose levels. Moreover, GLP-1 receptor agonists reduce appetite, gastric emptying rate, and bodyweight, whereas both antihyperglycemic drug classes improve blood pressure and (postprandial) lipid profiles in patients with type 2 diabetes mellitus.

The microcirculation encompasses all vessels of <150 μm, which includes arterioles, capillaries, and venules, and is involved in regulation of tissue perfusion to optimize nutrient delivery. An increase in microvascular perfusion improves glucose and insulin supply to the muscle interstitium, thereby enhancing peripheral glucose disposal. In addition, the microcirculation regulates peripheral vascular resistance, which in conjunction with cardiac output determines arterial blood pressure.

GLP-1 peptide has been shown to improve microvascular function. As such, GLP-1 infusion increased microvascular perfusion of skeletal muscle in rodents, leading to increased glucose disposal. Infusion of GLP-1 peptide has also been shown to improve microvascular perfusion in healthy humans, although this was not associated with glucose disposal in one study.

Evidence about the effects of GLP-1 receptor agonists or dipeptidyl peptidase-4 inhibitors on the microcirculation,
however, is scarce. In Wistar rats, 4-week treatment with the GLP-1 receptor agonist liraglutide or the dipeptidyl peptidase-4 inhibitor sitagliptin blunted the inhibiting effects of a high-fat diet on microvascular perfusion.\(^\text{10}\) Moreover, we recently demonstrated that acute administration of the GLP-1 receptor agonist exenatide increases microvascular blood flow, independent of NO availability, in healthy overweight men.\(^\text{11}\) However, to date, it is unknown whether GLP-1–based therapies affect microvascular perfusion in type 2 diabetes mellitus, a disease that is characterized by reduced microvascular function.\(^\text{12}\) Therefore, we assessed the effects of acute exenatide administration and 12-week treatment with liraglutide or sitagliptin on microvascular function in patients with type 2 diabetes mellitus. Given the beneficial effects of GLP-1–based therapies on glucose levels and blood pressure in this population, we hypothesized that these agents increase microvascular perfusion that contributes to these beneficial effects.

Materials and Methods

In the current study, 60 patients with type 2 diabetes mellitus underwent 2 randomized, double-blind, placebo-controlled trials, as described previously\(^\text{11}\): an acute intervention study to assess the effects of intravenous exenatide administration and a 12-week double dummy intervention study to assess the effects of liraglutide 1.8 mg daily or sitagliptin 100 mg daily treatment. In the fasting and postprandial state, microvascular function was assessed using nailfold skin capillary videomicroscopy and laser Doppler fluxmetry. A complete description of Materials and Methods is available in the online-only Data Supplement.

Results

Patient Characteristics

Baseline characteristics and study flow diagram are displayed in Figure 1. Three patients withdrew their consent during the run-in period, and 57 patients were randomized for the acute intervention study (exenatide n=29; placebo n=28). Before randomization for the long-term study, 1 patient was excluded because of incidental findings. Because of adverse effects, 1 patient dropped out in the sitagliptin arm (pollakiuria and dizziness). Thus, analyses were performed in 55 patients for the long-term study (liraglutide n=19, sitagliptin n=19, and placebo n=17).

Acute Intervention Study: Fasting State

Compared with placebo, acute intervention with exenatide infusion did not affect baseline (+1.0±1.2 capillaries/mm\(^2\); \(P=0.39\)) or postocclusive capillary density (+1.8±1.3 capillaries/mm\(^2\); \(P=0.18\); Table 1 and Figure 2). Exenatide did not affect total vasomotion, nor power in the cardiac, respiratory, and endothelial domains, compared with placebo \((P>0.05\); Table 1 and Figure 3). However, exenatide decreased power in the myogenic domain (log value: 0.05±0.03; \(P=0.05\)) and increased power in the neurogenic domain (log value: 0.04±0.01; \(P=0.004\)).

Compared with placebo, exenatide increased systolic blood pressure (SBP) by 7.3±3.5 mm Hg \((P=0.04)\), diastolic blood pressure (DBP) by 2.9±1.5 mm Hg \((P=0.05)\), and heart rate (HR) by 4.8±1.6 beats/minute \((P<0.01\); Table 1). Moreover, at the start of the microvascular measurements, exenatide decreased glucose levels by 1.6±0.1 mmol/L \((P<0.001)\) compared with placebo (Figure 4).

Acute Intervention Study: Postprandial State

During placebo infusion, the mixed meal did not affect baseline or postocclusion capillary density compared with fasting measurements (Table 1). However, meal ingestion induced an increase in power in the neurogenic vasomotion domain \((P=0.018)\).

Compared with placebo, exenatide did not affect baseline (+0.03±1.5 capillaries/mm\(^2\); \(P=0.99\)) or postocclusion capillary density (+1.7±2.4 capillaries/mm\(^2\); \(P=0.49\)) in the postprandial state (Table 1 and Figure 2). Moreover, exenatide increased endothelial domain power (log value: 0.05±0.02; \(P=0.01\)), without affecting other domains (Table 1 and Figure 3).

After the meal, SBP (12.3±3.6 mm Hg; \(P=0.001\)) and DBP (+8.1±1.6 mm Hg; \(P=0.001\)) increased with exenatide compared with placebo (Table 1). The glucose level at the start of the postprandial microvascular measurements with placebo was 9.9±0.2 mmol/L, whereas this was 4.2±0.3 mmol/L lower with exenatide \((P<0.001\); Figure 4).

12-Week Intervention Study: Fasting State

Compared with placebo, 12-week intervention with neither liraglutide (+1.6±2.5 capillaries/mm\(^2\); \(P=0.506\)) nor sitagliptin (-0.26±2.5 capillaries/mm\(^2\); \(P=0.916\)) affected baseline capillary density (Figure 2). Moreover, we observed no effects on postocclusion capillary density (liraglutide: 2.9±2.9 capillaries/mm\(^2\); \(P=0.330\); sitagliptin: +1.8±1.3 capillaries/mm\(^2\); \(P=0.184\)). Liraglutide and sitagliptin did not alter total vasomotion or specific domains, compared with placebo (Table 2 and Figure 3).

After 12-week treatment, liraglutide reduced SBP by 11.5±4.7 mm Hg \((P=0.02)\) and increased HR by 5.8±2.0 beats/minute \((P=0.006)\), compared with placebo (Table 2). Sitagliptin had no significant effect on SBP, DBP, or HR. Liraglutide reduced fasting glucose concentrations (1.3±0.3 mmol/L; \(P<0.001\)) and HbA\(_{\text{lc}}\) (glycated hemoglobin; 1.7±0.9%; \(P<0.001\)), compared with placebo. Sitagliptin reduced fasting glucose concentrations by 1.1±0.4 mmol/L \((P=0.003)\) and HbA\(_{\text{lc}}\) by 0.8±0.9% \((P=0.001)\).

12-Week Intervention Study: Postprandial State

Capillary density was similar between all treatment groups in the postprandial state (Table 2 and Figure 2). Moreover, no effect was observed on total vasomotion or specific domains of vasomotion (Figure 3).

After the meal, SBP reduced by 15.6±2.5 mm Hg with liraglutide and by 12.1±5.5 with sitagliptin (Table 2), compared with placebo, whereas DBP and HR remain unaffected. The glucose level at the start of the microvascular measurements...
with placebo was 10.3±0.4 mmol/L, whereas this was reduced by 1.3±0.5 mmol/L during both liraglutide and sitagliptin (P<0.01; Figure 4).

Correlations
In the acute fasting setting, the treatment-induced effect on vasomotion neurogenic power correlated with glucose (R=−0.502; P<0.001), DBP (R=+0.328; P=0.030), and HR (R=+0.350; P=0.023), after correction for body mass index and age (Figure I in the online-only Data Supplement). No other correlations between microvascular measurements and glucometabolic or hemodynamic factors were observed.

Discussion
This is the first study to assess the effects of GLP-1–based therapies on capillary perfusion in patients with type 2 diabetes mellitus.

### Table 1. Acute Effects of Exenatide on Parameters of Nailfold Skin Capillary Videomicroscopy, Laser Doppler Fluxmetry, and Systemic Hemodynamics in Type 2 Diabetes Mellitus

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo Pretreatment</th>
<th>Placebo Acute</th>
<th>Placebo Acute PP</th>
<th>Exenatide Pretreatment</th>
<th>Exenatide Acute</th>
<th>Exenatide Acute PP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nailfold skin capillary videomicroscopy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline, capillaries/mm²</td>
<td>44.0±1.7</td>
<td>49.4±1.7</td>
<td>51.0±1.5</td>
<td>40.9±1.3</td>
<td>46.5±1.5</td>
<td>48.2±1.5</td>
</tr>
<tr>
<td>Postocclusion, capillaries/mm²</td>
<td>58.2±1.9</td>
<td>62.1±1.8</td>
<td>62.5±1.6</td>
<td>54.8±1.9</td>
<td>59.8±2.3</td>
<td>60.6±2.6</td>
</tr>
<tr>
<td>Hyperemia, %</td>
<td>32.9±1.6</td>
<td>26.8±2.2</td>
<td>22.9±1.4</td>
<td>34.3±2.7</td>
<td>28.3±2.4</td>
<td>25.1±2.7</td>
</tr>
<tr>
<td>Laser Doppler fluxmetry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total power, ms²</td>
<td>97.4 (57.7–184.1)</td>
<td>93.2 (65.4–164.8)</td>
<td>113.4 (65.5–176.1)</td>
<td>68.7 (58.4–107.6)</td>
<td>97.3 (47.1–196.5)</td>
<td>136.4 (69.1–187.4)</td>
</tr>
<tr>
<td>Cardiac, NU</td>
<td>0.32 (0.22–0.44)</td>
<td>0.28 (0.23–0.40)</td>
<td>0.25 (0.17–0.33)</td>
<td>0.39 (0.31–0.47)</td>
<td>0.24 (0.19–0.31)</td>
<td>0.23 (0.20–0.33)</td>
</tr>
<tr>
<td>Respiratory, NU</td>
<td>0.26 (0.23–0.32)</td>
<td>0.30 (0.24–0.40)</td>
<td>0.35 (0.27–0.40)</td>
<td>0.30 (0.25–0.35)</td>
<td>0.29 (0.24–0.32)</td>
<td>0.31 (0.27–0.33)</td>
</tr>
<tr>
<td>Myogenic, NU</td>
<td>0.74 (0.62–0.81)</td>
<td>0.75 (0.68–0.91)</td>
<td>0.79 (0.70–0.94)</td>
<td>0.68 (0.55–0.76)</td>
<td>0.67 (0.60–0.78)</td>
<td>0.74 (0.63–0.79)</td>
</tr>
<tr>
<td>Neurogenic, NU</td>
<td>1.56 (1.48–1.73)</td>
<td>1.56 (1.46–1.61)</td>
<td>1.70 (1.57–1.80)</td>
<td>1.54 (1.43–1.63)</td>
<td>1.67 (1.53–1.74)*</td>
<td>1.59 (1.48–1.71)</td>
</tr>
<tr>
<td>Endothelial, NU</td>
<td>2.47 (2.29–2.75)</td>
<td>2.44 (2.28–2.73)</td>
<td>2.37 (2.14–2.68)</td>
<td>2.45 (2.32–2.79)</td>
<td>2.62 (2.17–2.87)</td>
<td>2.68 (2.43–2.87)*</td>
</tr>
<tr>
<td>Systemic hemodynamics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>136.1±3.0</td>
<td>143.9±3.8</td>
<td>137.0±3.1</td>
<td>135.5±2.6</td>
<td>151.3±3.2*</td>
<td>148.8±3.6*</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>76.2±1.4</td>
<td>80.2±1.4</td>
<td>77.3±3.6</td>
<td>76.3±1.3</td>
<td>83.4±1.7</td>
<td>82.8±1.6*</td>
</tr>
<tr>
<td>HR, BPM</td>
<td>66.5±1.9</td>
<td>65.9±2.1</td>
<td>67.6±2.2</td>
<td>63.2±1.2</td>
<td>67.5±1.5*</td>
<td>74.5±1.8</td>
</tr>
</tbody>
</table>

Nailfold skin capillary videomicroscopy and hemodynamic data are presented as crude mean±SEM; laser Doppler Fluxmetry data are presented as crude median (interquartile range). DBP indicates diastolic blood pressure; HR, heart rate; NU, normalized units; PP, postprandial; and SBP, systolic blood pressure.

* A significant difference (P<0.05) of treatment compared with placebo, corrected for potential differences in pretreatment values.
diabetes mellitus. For a comprehensive assessment, measurements were performed using 2 complementary techniques, capillary videomicroscopy and laser Doppler fluxmetry, after both an acute and 12-week intervention, in both the fasting and postprandial states. In none of the settings, we observed changes in capillary perfusion. However, in the acute setting, exenatide infusion increased fasting neurogenic and reduced myogenic power domains, whereas in the postprandial state, the drug increased endothelial domain power.

Nailfold skin capillary videomicroscopy was used to assess microvascular perfusion. Perfusion of capillaries is regulated by alternating the diameter of precapillary arterioles. Many mediators, including insulin and NO, are known to cause vasodilation of precapillary arterioles, leading to an increase in capillary perfusion. In animal studies, GLP-1 peptide infusion increases muscle microvascular perfusion. In healthy volunteers, infusion of GLP-1 improves microvascular perfusion in muscle tissue. We recently demonstrated that exenatide increased skin capillary perfusion in healthy men, using the same techniques and a similar study protocol as used in the current study. It was therefore unexpected to find no effect of exenatide on skin capillary perfusion in patients with type 2 diabetes mellitus in the current study. Such a differential effect in microvascular activity is also known for insulin, which improves microvascular perfusion in healthy volunteers, but has no effect on microvascular function in patients with type 2 diabetes mellitus. This discrepancy is attributed to dysfunction of the endothelium in type 2 diabetes mellitus. Potentially, this mechanism also explains the differential findings with GLP-1 and related therapies. Notably, vascular GLP-1 resistance is present in Ossabaw miniature swine with metabolic syndrome, but not in lean, healthy animals.

In the acute setting, exenatide significantly decreased power in the myogenic domain (P=0.049) and increased power in the neurogenic domain (P=0.004). In the acute study, exenatide increased power in the endothelial domain (P=0.01) after the meal. No differences were seen in the 12-week study. Data are presented as median with interquartile range. pp indicates postprandial.
The microcirculation is present throughout the body, and its physiological role differs depending on the studied organ. For example, muscle microvascular perfusion is involved in muscle glucose uptake.\textsuperscript{20} The microcirculation of the skin is largely involved in thermoregulation, by opening and closing of arteriovenous anastomoses.\textsuperscript{21} However, because glucose uptake in skin during hyperinsulinemia contributes significantly to whole body glucose uptake,\textsuperscript{22} we used capillary videomicroscopy to assess capillary perfusion in a noninvasive way. This allowed us to assess not only capillary perfusion but also functional capillary reserve.\textsuperscript{23} By doing so, we and others have repeatedly demonstrated that dorsal nailfold skin microcirculation (capillary density and capillary reserve) is associated with changes in glucose metabolism,\textsuperscript{13,24,25} in a much similar fashion as muscle microcirculation. However, although insulin-induced changes in skin and muscle microvascular perfusion are correlated,\textsuperscript{13} it must be repeated that these vascular beds serve different functions and may therefore respond differently to GLP-1–based therapies. Because glucose levels decreased in both the acute and 12-week studies, without changes in skin microvascular perfusion, future studies should assess whether GLP-1–based therapies affect muscle microcirculation.

Laser Doppler fluxmetry was performed to measure skin microvascular vasomotion, the rhythmic contraction and dilation of arterioles caused by changes in contraction and relaxation of smooth muscle cells in the vessel walls. Vasomotion is considered to be involved in several microvascular aspects, including tissue oxygenation, vascular resistance, maintenance of capillary pressure, and perfusion.\textsuperscript{26,27} Infusion of insulin leads to an increase in both total vasomotion and capillary perfusion, thereby likely allowing for increased glucose and insulin delivery to target cells.\textsuperscript{28} Moreover, using component analysis, distinct periodic oscillations in the laser Doppler vasomotion signal have been attributed to cardiac, respiratory, and vessel myogenic activity; neurogenic activity; and endothelial activity. As such, the laser Doppler signal can be used for total vasomotion per se, but also to assess aspects of neurogenic and endothelial activity.

Although we did not observe effects on total vasomotion, exenatide acutely increased activity in the neurogenic domain, which reflects an increase in microcirculatory sympathetic nervous system (SNS) activity.\textsuperscript{29} This is in line with previous findings, in which GLP-1 receptor agonists were shown to stimulate SNS activity, measured by heart rate variability,\textsuperscript{30,31} or plasma catecholamine levels.\textsuperscript{32} Although increased SNS activity is commonly associated with vasoconstriction, several studies have demonstrated that for the microcirculation, SNS activity might be associated with improved perfusion and consequently glucose uptake.\textsuperscript{25} For example, in healthy volunteers, clamped hyperinsulinemia increased power in the neurogenic domain, which was associated with increased capillary perfusion and glucose uptake.\textsuperscript{25} In line with these findings, here, the exenatide-induced increase in neurogenic power was strongly associated with reduced glucose levels, indicating a SNS-induced increase in glucose uptake. However, this was not accompanied by changes in capillary perfusion. Whether increased neurogenic activity could lower glucose without increasing skin capillary density is unknown, yet differences may exist between healthy volunteers and overweight patients with type 2 diabetes mellitus. Potentially, as discussed above, skin microcirculation does not adequately represent muscle microcirculation, where the largest part of glucose utilization occurs. Interestingly, in a recent study in rats and mice, stimulation of \(\beta_2\)-adrenoreceptors in skeletal muscle cells increased glucose uptake independent of the
Table 2. Effects of 12-Wk Treatment With Liraglutide or Sitagliptin on Parameters of Nailfold Skin Capillary Videomicroscopy, Laser Doppler Fluxmetry, and Systemic Hemodynamics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo Pretreatment</th>
<th>Placebo 12 Wk</th>
<th>Placebo 12-Wk PP</th>
<th>Sitagliptin Pretreatment</th>
<th>Sitagliptin 12 Wk</th>
<th>Sitagliptin 12-Wk PP</th>
<th>Liraglutide Pretreatment</th>
<th>Liraglutide 12 Wk</th>
<th>Liraglutide 12-Wk PP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nailfold Skin Capillary Videomicroscopy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline, capillaries/mm²</td>
<td>44.9±2.1</td>
<td>46.1±2.3</td>
<td>50.7±2.7</td>
<td>41.3±1.8</td>
<td>43.3±2.2</td>
<td>48.0±1.9</td>
<td>41.1±1.9</td>
<td>45.0±2.0</td>
<td>49.4±1.9</td>
</tr>
<tr>
<td>Postocclusion, capillaries/mm²</td>
<td>60.3±2.6</td>
<td>59.1±2.7</td>
<td>62.5±2.7</td>
<td>54.9±2.4</td>
<td>55.4±2.9</td>
<td>59.0±2.3</td>
<td>53.9±2.4</td>
<td>58.4±2.8</td>
<td>61.8±2.6</td>
</tr>
<tr>
<td>Hyperemia, %</td>
<td>35.1±2.9</td>
<td>29.1±3.3</td>
<td>25.1±3.5</td>
<td>33.3±2.7</td>
<td>28.5±2.8</td>
<td>23.4±2.4</td>
<td>31.9±2.7</td>
<td>29.4±2.3</td>
<td>24.8±2.1</td>
</tr>
<tr>
<td><strong>Laser Doppler fluxmetry</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total power, ms²</td>
<td>83.7 (56.8–161.6)</td>
<td>119.6 (64.5–173.4)</td>
<td>96.5 (59.5–153.1)</td>
<td>96.9 (59.9–185.4)</td>
<td>105.8 (67.4–164.8)</td>
<td>118.7 (87.7–186.4)</td>
<td>66.0 (56.7–127.1)</td>
<td>85.8 (66.9–141.2)</td>
<td>68.0 (46.8–142.3)</td>
</tr>
<tr>
<td>Cardiac, NU</td>
<td>0.40 (0.26–0.52)</td>
<td>0.29 (0.19–0.47)</td>
<td>0.28 (0.17–0.45)</td>
<td>0.31 (0.22–0.44)</td>
<td>0.35 (0.26–0.37)</td>
<td>0.22 (0.17–0.32)</td>
<td>0.40 (0.33–0.45)</td>
<td>0.29 (0.19–0.37)</td>
<td>0.25 (0.19–0.36)</td>
</tr>
<tr>
<td>Respiratory, NU</td>
<td>0.25 (0.20–0.29)</td>
<td>0.28 (0.25–0.38)</td>
<td>0.29 (0.28–0.37)</td>
<td>0.28 (0.25–0.34)</td>
<td>0.32 (0.24–0.40)</td>
<td>0.31 (0.24–0.36)</td>
<td>0.32 (0.25–0.39)</td>
<td>0.32 (0.26–0.39)</td>
<td>0.31 (0.26–0.40)</td>
</tr>
<tr>
<td>Myogenic, NU</td>
<td>0.67 (0.50–0.78)</td>
<td>0.64 (0.54–0.78)</td>
<td>0.77 (0.67–0.88)</td>
<td>0.71 (0.59–0.78)</td>
<td>0.72 (0.62–0.84)</td>
<td>0.78 (0.59–0.90)</td>
<td>0.73 (0.64–0.87)</td>
<td>0.77 (0.65–0.92)</td>
<td>0.81 (0.69–0.83)</td>
</tr>
<tr>
<td>Neurogenic, NU</td>
<td>1.51 (1.42–1.63)</td>
<td>1.57 (1.43–1.69)</td>
<td>1.65 (1.47–1.75)</td>
<td>1.55 (1.43–1.64)</td>
<td>1.56 (1.51–1.66)</td>
<td>1.67 (1.62–1.81)</td>
<td>1.57 (1.49–1.66)</td>
<td>1.61 (1.47–1.72)</td>
<td>1.62 (1.51–1.73)</td>
</tr>
<tr>
<td>Endothelial, NU</td>
<td>2.49 (2.35–2.99)</td>
<td>2.46 (2.32–2.88)</td>
<td>2.52 (2.17–2.65)</td>
<td>2.46 (2.34–2.80)</td>
<td>2.55 (2.28–2.74)</td>
<td>2.38 (1.86–2.83)</td>
<td>2.36 (2.02–2.58)</td>
<td>2.51 (2.09–2.69)</td>
<td>2.49 (2.31–2.67)</td>
</tr>
<tr>
<td><strong>Systemic hemodynamics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>137.6±3.9</td>
<td>139.7±3.2</td>
<td>148.0±4.9</td>
<td>132.5±2.8</td>
<td>132.1±3.1</td>
<td>134.6±3.2*</td>
<td>136.6±3.9</td>
<td>127.5±4.9</td>
<td>132.8±3.9*</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>76.4±1.5</td>
<td>76.2±1.5</td>
<td>75.5±2.0</td>
<td>76.9±1.2</td>
<td>76.2±2.4</td>
<td>74.7±2.1</td>
<td>76.9±1.2</td>
<td>76.2±2.4</td>
<td>74.7±2.1</td>
</tr>
<tr>
<td>HR, BPM</td>
<td>64.5±2.2</td>
<td>64.1±2.5</td>
<td>74.7±3.1</td>
<td>63.0±1.8</td>
<td>64.1±1.6</td>
<td>71.6±2.4</td>
<td>66.7±2.1</td>
<td>71.4±1.7</td>
<td>80.2±2.3</td>
</tr>
</tbody>
</table>

Nailfold skin capillary videomicroscopy and hemodynamic data are presented as crude mean±SEM. Laser Doppler fluxmetry data are presented as crude median (interquartile range). DBP indicates diastolic blood pressure; HR, heart rate; NU, normalized units; PP, postprandial; and SBP, systolic blood pressure.

*A significant difference (P<0.05) of treatment compared with placebo, corrected for potential differences in pretreatment values.

Insulin signaling pathway, which can explain the lack of effect on microvascular perfusion. Because we only measured glucose levels, and not muscle glucose uptake, additional studies are needed to assess whether the inverse association between neurogenic activity and glucose levels is mediated by increased glucose uptake.

Interestingly, not all studies are suggestive of increased SNS activity after GLP-1 receptor agonist intervention. For example, we recently demonstrated that exenatide infusion decreased neurogenic activity in healthy overweight men. In that study, the effect on neurogenic activity was negatively correlated with capillary density, leading us to speculate that an exenatide-induced reduction in dermal SNS activity reduces vascular tone and consequently increases capillary density.

Increased neurogenic activity was accompanied by reduced myogenic power, although the latter was statistically rather weak and might be a false-positive finding. Nevertheless, reduced myogenic power is known to occur with vasoconstriction. When combined, 2 distinct mechanisms could be proposed: (1) acute exenatide administration primarily increases SNS activity, leading to vasoconstriction and increased blood pressure; (2) acute exenatide administration increases blood pressure and as a consequence, autoregulatory vasoconstrictive responses maintain stable microvascular perfusion. Exenatide-induced reductions in glucose levels could contribute to both mechanisms, because low glucose levels increase SNS activity and blood pressure; however, none of the subjects experienced symptoms of hypoglycemia.

The acute increase in SNS activity with exenatide could have detrimental cardiovascular and metabolic effects when sustained. However, after 12-week intervention with liraglutide or sitagliptin, no SNS increase was observed. This difference could be explained by differences in pharmacodynamics and pharmacokinetics, as is well known with GLP-1–based therapies. For example, although the short-acting GLP-1 receptor agonist exenatide reduces gastric emptying rate, this does not occur with the long-acting GLP-1 receptor agonist liraglutide or sitagliptin. Alternatively, the effects seen with acute intervention wane over time.

Microvascular perfusion and vasomotion are known to increase after meal ingestion, likely to increase the available endothelial surface allowing a more efficient (muscle) glucose disposal. In obesity, this postprandial mechanism is blunted, which is likely caused by insulin resistance and endothelial dysfunction, and associates with impaired postprandial glucose metabolism. In accordance with these previous

Downloaded from http://atvb.ahajournals.org/ by guest on August 25, 2017
GLP-1–Based Therapies and Microcirculation

Smits et al.

studies, we observed no effect of the meal on capillary perfusion in the current study. Interestingly, infusion of exenatide increased power in the endothelial domain of vasomotion. This corresponds with the observation that a single subcutaneous dose of exenatide prevents postprandial endothelial dysfunction in subjects with prediabetes or diabetes mellitus,\textsuperscript{29,40} when measured by reactive hyperemia applanation tonometry. However, because we observed no exenatide changes in capillary perfusion or total vasomotion in current trial, it is unclear whether the improvement in endothelial power has physiological relevance.

The current study has some limitations. First, studies were not performed during (pancreas) clamp procedures, and as such, the effects of GLP-1–based therapies on insulin and other hormones could have influenced microvascular measurements. This may also have occurred in the 12-week treatment interventions and placebo. However, although this obscures interpretation of the direct effects of GLP-1 on microvascular function, the results represent real-life effects of these therapeutic agents. Second, we did not perform glucose tracer studies, which would have allowed us to assess the effects of GLP-1–based therapies on muscle glucose uptake. Third, as stated, microvascular assessments were a secondary end point of a larger study,\textsuperscript{11} and therefore, the sample size was not based on calculations for this end point. Nevertheless, using data from our previous study in healthy overweight volunteers, a sample size of 15 patients per group was needed, indicating that the a priori sample size was sufficient to detect clinically relevant changes. Finally, including a treatment arm with overweight healthy volunteers would have been interesting, given the recent approval of liraglutide for weight loss treatment.\textsuperscript{41}

In conclusion, despite acute exenatide-induced increases in fasting skin SNS activity and postprandial endothelial function, no effect on capillary perfusion was observed in patients with type 2 diabetes mellitus. Moreover, 12-week treatment with liraglutide or sitagliptin did not affect capillary perfusion or vasomotion in these patients. Our data suggest that the effects of GLP-1–based therapies on plasma glucose and blood pressure levels are not caused by changes in microvascular function.

Acknowledgments

We thank all participants for their time and commitment to the demanding research protocol. Furthermore, we would like to thank J. Boerop for her excellent practical assistance during the visits. M.M.S. developed the study protocol, performed the measurements and analyses, and wrote the article. L.T. performed measurements and wrote the article. M.D. developed the study protocol and was involved in the discussion. M.H.A.M., T.H., M.H.H.K., E.H.S., and D.H.V.R. contributed to the discussion and edited the article.

Sources of 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. Liraglutide and liraglutide placebo were kindly provided by Novo Nordisk A/S (Bagsværd, Denmark). Funders had no role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Disclosures

Through M.H.H.K. and M.D., the VU University Medical Center received research grants from AstraZeneca, Boehringer Ingelheim, Novo Nordisk, and Sanofi. The other authors report no conflicts.

References


Exenatide acutely increases activity in the neurogenic domain of vasomotion, representing increased activity of the sympathetic nervous system, yet this effect is not seen after 12-week intervention. In the postprandial state, exenatide acutely increased activity in the endothelial domain of vasomotion, indicating improved endothelial function, yet this effect is not seen after 12-week intervention.
GLP-1–Based Therapies Have No Microvascular Effects in Type 2 Diabetes Mellitus: An Acute and 12-Week Randomized, Double-Blind, Placebo-Controlled Trial
Mark M. Smits, Lennart Tonneijck, Marcel H.A. Muskiet, Trynke Hoekstra, Mark H.H. Kramer, Michaela Diamant, Erik H. Serné and Daniël H. van Raalte

Arterioscler Thromb Vasc Biol. 2016;36:2125-2132; originally published online August 25, 2016;
doi: 10.1161/ATVBAHA.116.307930
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2016 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/36/10/2125

Data Supplement (unedited) at:
http://atvb.ahajournals.org/content/suppl/2016/08/25/ATVBAHA.116.307930.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/
Materials and Methods

In the current study, 60 patients with type 2 diabetes underwent two randomized, double-blind, placebo-controlled trials, as described previously: an acute intervention study to assess the effects of intravenous exenatide administration; and a 12-week double-dummy intervention study to assess the effects of liraglutide 1.8 mg daily or sitagliptin 100 mg daily treatment. Tests were performed in the fasting and after a standardized mixed meal. These postprandial measurements were deemed important since 1) humans reside most of their time in the postprandial state; 2) GLP-1 receptor agonists stimulate glucose-stimulated insulin secretion and reduce gastric emptying, and as such, might have different effects after meal ingestion; and 3) DPP-4 inhibitors reduce the degradation of endogenously released GLP-1; these agents may be more active after meal ingestion with subsequent GLP-1 release.

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 participants provided written informed consent before participation.

Figure Supplemental I: Study design

A) Acute intervention study

\[
\text{Exenatide or Placebo} \quad \text{Meal} \quad \text{Exenatide or Placebo}
\]

\[
\begin{array}{c}
\text{measurements} \\
(\text{min}) \quad -200 \quad 0 \quad 30 \quad 120 \quad 150 \quad 175 \quad 265 \quad 300 \\
\end{array}
\]

B) 12-week intervention study

\[
\text{Liraglutide 1.8 mg once daily} \\
\text{Sitagliptin 100 mg once daily} \\
\text{Placebo once daily}
\]

\[
\begin{array}{c}
\text{measurements} \\
(\text{wk}) \quad 0 \quad 12 \quad 0 \quad (\text{min}) \quad 90 \quad 120 \\
\end{array}
\]

Legend: Study design of the (A) acute intervention study and (B) 12-week intervention study. The grey areas indicates measurement of microvascular function. No baseline postprandial measurements were performed for the 12-week study.
Participants
We recruited Caucasian males and postmenopausal females with type 2 diabetes, aged 35 to 75 years old, with an HbA1c of 6.5 to 9.0% (48-75 mmol/mol) and BMI of 25 to 40 kg/m². Patients were treated with a stable dose of metformin and/or sulfonylurea derivatives for at least 3 months. Major exclusion criteria included treatment with insulin or GLP-1 based therapy at time of inclusion, history of pancreatic, hepatic, cardiovascular, or renal disease, an estimated glomerular filtration rate <60 mL/min/1.73m², and allergy to any of the test substances.

Acute study
First, pre-treatment endpoint measurements were performed for both the acute and 12-week intervention study. Thereafter, participants were randomized by the trial pharmacist using computer-generated numbers (block-size of 6; allocation ratio of 1:1), to receive intravenous exenatide (AstraZeneca, London, UK) or placebo (saline 0.9%). As validated previously by our research group and others, an exenatide loading dose of 50 ng/min for 30 minutes, followed by a continuous infusion rate of 25 ng/min, yields stable plasma levels within the therapeutic range and harbors a good tolerability profile. The intravenous route was preferred over the subcutaneous route, since this allowed us to perform a blinded study in the absence of exenatide-placebo administration pens. Endpoint measurements were repeated 120 minutes after the start of the intervention. Subsequently, a high-carbohydrate, high-fat, mixed meal was administered (905.7 kCal; 50 g fat, 75 g carbohydrates and 36.8 g protein), and endpoint measurements were repeated after 90 minutes in the postprandial state.

12-week study
After the acute study, subjects were randomized by the trial pharmacist using computer-generated numbers (block-size of 6; allocation ratio of 1:1:1; stratified to either 2 interventions of the acute study) to receive liraglutide (Novo Nordisk A/S, Bagsværd, Denmark), sitagliptin (Merck & Co., Kenilworth, NJ, USA), or matching placebos (all subjects received pens for subcutaneous injections and oral capsules). Novo Nordisk A/S provided pre-filled pens with either liraglutide or placebo, while ACE Pharmaceuticals (Zeewolde, The Netherlands) encapsulated the sitagliptin or placebo tablets. Participants randomized to the liraglutide-arm received dummy capsules, participants randomized to the sitagliptin-arm received dummy pre-filled pens, while the placebo-arm received dummy capsules and pens (double-dummy design). Study drugs were taken once-daily, at the same time of day 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; remaining weeks: 1.8 mg daily). Based on tolerance to the subcutaneous study drug, time between dose increments could be extended, and maximal administration dose could be reduced, based on the discretion of the investigators. After 12 weeks of intervention, the endpoint measurements were repeated. Moreover, an equivalent mixed meal as used for the acute study was administered, and endpoint microvascular measurements were repeated after 90 minutes in the postprandial state.
Study End Points
Two days prior to the study visits, subjects were instructed to adhere to a normal-salt (9-12 grams per day) and normal-protein (1.5-2 mg/kg per day) diet. In addition, prior to the study visit, all participants abstained from heavy exercise and alcohol for 24 hours and caffeine for 12 hours. Subjects arrived at the Diabetes Center of the VU University Medical Center at 07.30 AM after an overnight fast. An intravenous catheter was placed in an antecubital vein, and subjects were instructed to assume a semi-recumbent position throughout the testing-day. After 30 minutes of acclimatization in a temperature-controlled room (23.0±1.0 °C), the study tests commenced. Skin temperature was registered continuously and was above 28 °C at the start of all microvascular measurements.

Nailfold skin capillary videomicroscopy was performed using the VCS Video Capillaroscopy System (KK Technology, Honiton, UK). This system includes a high-quality monochrome CCD camera (resolution 752 x 582 pixels), and uses cold light epi-illumination for high-contrast images. The microscope was coupled to a laptop running CapiScope software version 3.90 (KK Technology, Honiton, UK) for image recording and analysis. The nailfold of the third digit of the non-dominant hand was placed under the microscope at the subjects' heart level. Two separate visual fields of 1 mm² were recorded before (baseline recordings) and after 4 minutes of arterial occlusion (established by inflating a cuff placed around the base of the finger to 300 mmHg). We aimed to identify and measure the same two fields for every assessment. Baseline recordings were analyzed for capillary density, which is the number of capillaries per mm² of nail fold skin that are continuously perfused for 15 seconds, representing functional capillary perfusion. The maximum number of capillaries counted directly after release of arterial occlusion defined peak capillary density, representing capillary reserve capacity. The investigator counting the capillaries (MMS) was blinded to the status of the recordings. Second time blinded counting established an intra-observer coefficient of variation of 3.9%.

A laser Doppler fluxmetry system (Periflux 4000; Perimed, Stockholm, Sweden) was used to measure vasomotion, which is a continuous oscillation of microvascular vessel diameter that contributes to tissue oxygenation, vascular resistance, and maintenance of capillary pressure. Thirty-minute recordings were acquired using a thermostatic laser Doppler probe (PF 481; Perimed, Stockholm, Sweden) placed at the dorsal side of the middle phalanx of the middle finger of the dominant hand. Wavelet analysis was performed using Matlab (Version 7.8.0.347; The Mathworks, Inc., Natick, MA, USA) to assess the frequency spectrum between 0.01 and 1.6 Hz. This spectrum was then divided into five frequency intervals: 0.01–0.02 Hz (endothelial activity); 0.02–0.06 Hz (neurogenic activity); 0.06–0.15 Hz (smooth muscle response in the vessel wall); 0.15–0.4 Hz (respiratory function); and 0.4–1.6 Hz (cardiac function). Since laser Doppler flux signal strength varies between subjects and between measurements, normalized amplitudes were calculated for each of the five frequency bands by dividing the average amplitude within a band by the average amplitude of the entire spectrum.

An automatic oscillometric device (Dinamap®, GE Healthcare, Little Chalfont, UK) was used to measure systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) at the non-dominant arm, using adequate cuff sizes. Measurements were performed
before the microvascular measurements, in triplicate at 1-2 min intervals, and the mean of the last 2 measurements was used for each time point.

Blood samples were drawn from the intravenous catheter. Venous plasma glucose was repeatedly assessed during measurements with a YSI 2300 STAT Glucose analyzer (YSI Life Sciences, Yellow Springs, Ohio, USA). HbA1c was measured using high-performance liquid chromatography.

**Statistics**

As current data are part of a larger study, of which the mechanistic effects on microvascular function were added as a secondary endpoint, no a-priori power calculation was performed for nailfold skin capillary videomicroscopy. However, when using the results of our study in healthy overweight volunteers, where exenatide increased capillary density by 20%, a sample size (with α 0.05; power 0.80) of 15 participants per group was calculated.

All data were double-entered into an electronic data management system (OpenClinica LLC, version 3.3, Waltham, MA, USA) and then exported to the study database. To test treatment effects versus placebo, regression models were used in the per-protocol population. For the acute intervention study, treatment with exenatide was added to the model. For the 12-week study, treatment with liraglutide or sitagliptin were both included as dummy-variable. Additionally, for both the acute and 12-week study, the pre-treatment value of the tested endpoint was included in the model as covariate, in order to correct for potential baseline differences (apart from the postprandial measurements in the 12-week study). In case variables demonstrated a non-Gaussian distribution, log-transformation was strived before analysis. Results from these analyzes are presented as treatment induced means ± standard error of the mean (SEM), corrected for placebo-effects and pre-treatment values. Given the physiologic relation between microvascular perfusion, vasomotion, systemic hemodynamics and glucose levels, we assessed correlations between treatment-induced changes in these variables. To correct these correlations for BMI and age, which are known to influence capillary perfusion and vasomotion, we performed linear regression analyses and report the standardized beta. Finally, we performed an exploratory analysis in placebo-treated patients in the acute study using paired T-tests (capillary videomicroscopy data) and Wilcoxon (vasomotion data), to assess the effects of meal-stimulation on microvascular function. All analyzes were performed using SPSS 22.0 (IBM SPSS Inc., Chicago, IL, USA), and a two-sided p-value of ≤0.05 was considered to be statistically significant. Correction for multiple testing was not performed in this hypothesis-generating study, since we feel that the risk of finding false-positive results is outweighed by the risk of missing potential mechanisms by stringent statistical correction techniques.
References


