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GLP-1 Is a Coronary Artery Vasodilator in Humans

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Background—The mechanism underlying the beneficial cardiovascular effects of the incretin GLP-1 (glucagon-like peptide 1) and its analogues in humans is elusive. We hypothesized that activating receptors located on vascular smooth muscle cells to induce either peripheral or coronary vasodilatation mediates the cardiovascular effect of GLP-1.

Methods and Results—Ten stable patients with angina awaiting left anterior descending artery stenting underwent forearm blood flow measurement using forearm plethysmography and post–percutaneous coronary intervention coronary blood flow measurement using a pressure-flow wire before and after peripheral GLP-1 administration. Coronary sinus and artery blood flows were sampled for GLP-1 levels. A further 11 control patients received saline rather than GLP-1 in the coronary blood flow protocol. GLP-1 receptor (GLP-1R) expression was assessed by immunohistochemistry using a specific GLP-1R monoclonal antibody in human tissue to inform the physiological studies. There was no effect of GLP-1 on absolute forearm blood flow or forearm blood flow ratio after GLP-1, systemic hemodynamics were not affected, and no binding of GLP-1R monoclonal antibody was detected in vascular tissue. GLP-1 reduced resting coronary transit time (mean [SD], 0.87 [0.39] versus 0.63 [0.27] seconds; P=0.02) and basal microcirculatory resistance (mean [SD], 76.3 [37.9] versus 55.4 [30.4] mm Hg/s; P=0.02), whereas in controls, there was an increase in transit time (mean [SD], 0.48 [0.24] versus 0.83 [0.41] seconds; P<0.001) and basal microcirculatory resistance (mean [SD], 45.9 [34.7] versus 66.7 [37.2] mm Hg/s; P=0.02). GLP-1R monoclonal antibody binding was confirmed in ventricular tissue but not in vascular tissue, and transmyocardial GLP-1 extraction was observed.

Conclusions—GLP-1 causes coronary microvascular dilation and increased flow but does not influence peripheral tone. GLP-1R immunohistochemistry suggests that GLP-1 coronary vasodilatation is indirectly mediated by ventricular-coronary cross talk.

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Key Words: coronary blood flow reserve • coronary microvascular function • coronary microvascular resistance • GLP-1 (glucagon-like peptide 1)

I

schemic heart disease resulting from coronary artery disease remains the leading cause of death globally. Type 2 diabetes mellitus is a significant cardiovascular risk factor and is becoming increasingly prevalent. Compared with the general population, patients with type 2 diabetes mellitus have a significantly increased risk of atherosclerotic coronary artery disease and myocardial infarction as well as a higher risk of developing heart failure and associated mortality.1–4 The poor prognosis of patients with type 2 diabetes mellitus is partially explained by the diffuse distribution of their coronary artery disease, as well as metabolic dysfunction that particularly affects the systemic microvasculature, including the heart.1,2,5 Improving the microcirculatory function in patients with type 2 diabetes mellitus could reduce morbidity and improve prognosis after myocardial infarction.6

Modulation of the GLP-1 (glucagon-like peptide 1) pathway is beneficial in the treatment of diabetes mellitus. Studies assessing the incretin hormone GLP-1 receptor (GLP-1R) agonists and dipeptidyl peptidase-4 (the enzyme responsible for GLP-1 [7–36] breakdown to metabolically inactive GLP-1 [9–36]) inhibitors, used to treat diabetes
mellitus, have confirmed that these classes of antihyperglycemic drugs do not appear to have deleterious cardiovascular effects,7–9 unlike other agents, and suggest that these agents may actually be cardioprotective.10–14 There are 2 studies to date, in which GLP-1R agonists liraglutide and semaglutide were associated with a reduction in myocardial infarction and cardiovascular death compared with placebo.7,15 The mechanism behind these nonglycemic effects is not clear, but the GLP-1R has been reported to be expressed in vascular smooth muscle cells16,17; and active GLP-1 (7–36) NH₂ and its metabolite (9–36) NH₂ are thought to have peripheral vasodilatory effects in animals.18–20 These vasodilatory effects may be beneficial cardioprotectively in patients with diabetes mellitus, although supporting data in humans are not extensive or consistent.21–25

We designed a mechanistic study to comprehensively evaluate the effects of GLP-1 (7–36) on both coronary and peripheral artery vascular tone in the same patients and to determine whether GLP-1 extraction occurred by measuring the transmyocardial gradient of GLP-1 (7–36) and GLP-1 (9–36) during GLP-1 (7–36) administration. We also assessed the pattern of GLP-1R expression by immunohistochemistry in human tissue samples, to support our physiological study findings.

Methods
The data, analytic methods, and study materials will not be made available to other researchers for purposes of reproducing the results or replicating the procedure, other than what is presented in this article.

Forearm and Coronary Blood Flow Protocols
Twenty-one patients with stable angina and a stenosis to their proximal left anterior descending coronary artery requiring stenting were identified at the cardiology preadmission clinic from the waiting list for elective percutaneous coronary intervention (PCI) at Royal Papworth Hospital between December 2015 and August 2017.

Patients receiving GLP-1–based therapy (GLP-1R agonist or dipeptidyl peptidase-4 inhibitor) were excluded to ensure baseline levels of GLP-1 were in the physiological range. Patients receiving insulin were also excluded to minimize the risk of hypoglycemia; and those taking calcium channel blockers, nicorandil, or oral nitrates had them discontinued 48 hours before assessment, because of the vasodilatory effects of these medications. The first 10 patients were invited to participate in both the forearm and coronary blood flow studies to assess the effect of GLP-1. A further 11 patients were block allocated as saline control patients in the coronary blood flow protocol.

The protocol was approved by the local ethics committee (Health Research Authority (HRA) Committee East of England, Research Ethics Committee (REC) reference 14/EE/0018), and written informed consent was obtained from all participants before they were enrolled into the study. The study was performed according to institutional guidelines, was registered on http://www.clinicaltrials.gov (unique identifier: NCT03502083), and was performed in accordance with the Declaration of Helsinki.

Forearm Blood Flow Study
All procedures were performed in a quiet and temperature-controlled room (23±2°C). Subjects were asked to refrain from alcohol, caffeinated beverages, and any vigorous exercise for 24 hours before the forearm blood flow study. The volunteers were encouraged to stay as still as possible throughout the forearm blood flow study.

A 27-gauge needle (Coopers Needleworks, Birmingham, UK) was inserted into the brachial artery, usually in the nondominant arm, under local anesthesia (1% lignocaine hydrochloride; Hamelin Pharmaceuticals Ltd). Venous occluding cuffs on both the upper arms were inflated to 40 to 45 mm Hg for 5 to 10 seconds to interrupt the venous flow, and arteriovenous communications in both the hands were removed by inflating the wrist cuffs to above systolic pressure (200–220 mm Hg). Basal and stimulated (after GLP-1) forearm blood flow responses were measured by mercury strain gauges, which act as resistors, placed at the widest part of both the forearms.26

Basal blood flow was measured after 15 minutes of saline infusion. A finger-prick blood glucose test result was measured at the start, and after each dose of GLP-1, if it...
was <3 mmol/L at any point, the study was terminated. Five weight-adjusted concentrations of GLP-1 (0.0375, 0.075, 0.15, 0.3, and 0.6 pmol/kg per minute) were then infused at a rate of 1 mL/min intra-arterially by an infusion pump (Alaris GH; Cardinal Health, Switzerland), each for 6 minutes; and forearm blood flow was measured during the last 3 minutes of each dose. Blood pressure in the noninfused arm was recorded by automated cuff at baseline, and after each dose of GLP-1, and heart rate was recorded by Electrocardiogram monitor at baseline and every minute during the GLP-1 infusions. GLP-1 was washed out with saline at the end of the study before removing the arterial needle. Patients were allowed to eat and drink, and their blood glucose was monitored for 2 hours before they were discharged.

Forearm blood flow data were analyzed using LabChart 8 (ADInstruments, Oxford, UK). The final 5 good-quality traces, usually recorded in the last minute of each 3-minute recording, were analyzed; and they were expressed as mean forearm blood flow in milliliters per minute per 100 mL forearm tissue, in the infused arm only, or as a ratio between infused and noninfused arm.27

**Coronary Blood Flow Study**

PCI was performed via radial or femoral arterial approach using 6F guide catheters. A 6F femoral venous sheath was inserted for central venous administration of adenosine and peripheral blood sampling. A multipurpose or Amplatz left catheter was positioned from the venous sheath into the right atrium for assessment of central venous pressure (Pc) and blood sampling from the coronary sinus. The coronary guide catheter provided access for coronary artery blood sampling.
All patients received aspirin, 300 mg, and clopidogrel, 300 mg, preloading. Patients were anticoagulated with a heparin bolus (70–100 U/kg) after arterial sheath insertion to achieve an activated clotting time >250 seconds. Iopromide (Ultravist; Bayer HealthCare Pharmaceuticals, Leverkusen, Germany) was used as the contrast agent for all cases. The choice of stent and implantation technique was left to operator discretion.

Pressure Wire Assessment

After proximal left anterior descending artery PCI, a PressureWire X (Abbott, Santa Clara, CA), linked wirelessly to Coroventis analysis software, was calibrated to atmospheric pressure before insertion and then advanced to the tip of the guiding catheter, where aortic and wire pressures were equalized. The wire was then advanced distally in the stented left anterior descending artery, with the pressure transducer positioned in the distal third of the coronary artery in a segment free from adjacent side branches; and this position was maintained throughout the study. A 0.2-mg bolus of intracoronary glyceryl trinitrate was administered, and once steady state was achieved, baseline coronary pressure and flow measurements were measured. The latter was derived from the reciprocal of transit time (Tmn) of an intracoronary injectate of room-temperature saline (thermodilution technique), as previously described. Coronary microcirculatory hyperemia was induced by intravenous administration of adenosine, 0.14 mg/kg per minute, into the femoral vein. At maximal hyperemia, Tmn, aortic pressure (Pa), and coronary distal pressure (Pd) were recorded (Figure 1). Coronary wedge pressure (Pw) was measured separately as Pd during occlusive balloon inflation within the stented segment. Measurements were recorded at baseline after stent deployment and after a 20-minute infusion of GLP-1 (7–36) via peripheral vein at a dose of 1.2 pmol/kg per minute (n=10) or an infusion of normal saline (control group, n=11) determined by block allocation. At the end of the procedure, the pressure wire was withdrawn to the coronary ostium to enable pressure-drift correction of Pd as necessary.

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Table 1. Patient Demographics

| Demographics           | GLP-1 Group (n=10) | Control Group (n=11) | P Value |
|------------------------|--------------------|----------------------|---------|
| Age, y                 | 68.3 (10.0)        | 61.8 (7.2)           | 0.11    |
| Male sex               | 8 (80)             | 11 (100)             | 0.21    |
| Weight, kg             | 88.4 (15.3)        | 91.2 (11.6)          | 0.63    |
| Height, m              | 1.71 (0.15)        | 1.76 (0.07)          | 0.33    |
| Body mass index, kg/m² | 30.2 (3.0)         | 29.7 (5.2)           | 0.79    |
| Comorbidity            |                    |                      |         |
| Pre–diabetes mellitus  | 1 (10)             | 2 (18)               | 1.00    |
| Hypertension           | 6 (60)             | 8 (73)               | 0.66    |
| Previous MI            | 1 (10)             | 8 (73)               | <0.01   |
| (Ex-) smoker           | 7 (70)             | 7 (64)               | 1.00    |
| Medications            |                    |                      |         |
| ACEI/ARB               | 3 (30)             | 7 (64)               | 0.20    |
| Statin                 | 10 (100)           | 11 (100)             | 1.00    |
| β-Blocker              | 4 (40)             | 8 (73)               | 0.20    |
| Blood                  |                    |                      |         |
| Total cholesterol, mg/dL | 178.7 (95.4)    | 160.7 (14.5)         | 0.54    |
| Creatinine, mg/dL      | 1.0 (0.2)          | 1.0 (0.2)            | 0.58    |
| Intervention           |                    |                      |         |
| Stent length, mm       | 36.7 (14.8)        | 36.5 (17.1)          | 0.97    |
| Stent diameter, mm     | 3.4 (0.6)          | 3.6 (0.5)            | 0.34    |
| Total balloon inflation time, s | 107.5 (39.8) | 119.2 (56.8) | 0.60 |

Data are given as mean (SD) or number (percentage). ACEI indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; GLP-1, glucagon-like peptide-1; MI, myocardial infarction.

Figure 2. No significant change in heart rate from baseline and every minute during the 6 infused doses of GLP-1 (glucagon-like peptide-1 [7–36]): 0.0375, 0.075, 0.15, 0.3, and 0.6 pmol/kg per minute; n=10.
These measurements enabled the calculation of the following: index of microcirculatory resistance (mm Hg/s or units) = \( \frac{P_a - P_w}{C_0 P_w} \) hyperemia corrected for collateral; basal microcirculatory resistance (BMR; mm Hg/s or units) = \( \frac{P_a - P_w}{C_0 P_w} \) baseline; resistance reserve ratio = BMR/index of microcirculatory resistance; coronary flow reserve (CFR) = \( \frac{T_{mn(base line)}}{T_{mn(hyperemia)}} \); and fractional flow reserve = \( \frac{P_a - P_v}{C_0 P_v} \) hyperemia.

Blood glucose was measured at baseline and again after the 20-minute infusion of GLP-1 to monitor and respond to any hypoglycemia. Heart rate and blood pressure were also recorded at baseline and after the 20-minute GLP-1 infusion. Once all measures had been collected, the GLP-1 infusion was terminated and patients resumed their usual clinical care.

Biochemistry

Paired blood samples were taken simultaneously from the coronary sinus, coronary artery, and peripheral vein at the end of the stent procedure and during the last minute of GLP-1 (7–36) infusion to measure GLP-1 (7–36), GLP-1 (9–36), insulin, free fatty acids, glucose, and lactate. Syringes for GLP-1 samples were prefilled with dipeptidyl peptidase-4 inhibitor (Millipore, UK) to prevent degradation. Intact GLP-1 (7–36) NH2 and the GLP-1 metabolite (9–36) NH2 were measured using a specific sandwich ELISA, as described previously.31,32

Immunohistochemistry

Finally, human tissue from anonymous donors, provided by the Royal Papworth Hospital Tissue Bank and the Cambridge Biorepository for Translational Medicine (http://www.cbtm.group.cam.ac.uk), was analyzed by immunohistochemistry to help us correlate our clinical findings with GLP-1R localization.
Tissue samples were fixed in 4% paraformaldehyde in 0.1 mol/L phosphate buffer for a minimum of 24 hours before dehydration and paraffin embedding. Pancreas was used as a positive control and stained with hematoxylin-eosin to identify the β cells. Tissue samples from pancreas, atria, ventricle, coronary artery, saphenous vein, left internal mammary artery, radial artery, and aorta underwent immunohistochemistry analysis using the monoclonal antibody (mAb) 3F52 GLP-1R antibody; this antibody was sourced from the University of Iowa Developmental Studies Hybridoma Bank and labelled with 3,3'-diaminobenzidine, which is oxidized in the presence of peroxidase and hydrogen peroxide, depositing a brown, alcohol-insoluble precipitate at the site of enzymatic activity. 17 mAb 3F52 was deposited to the Developmental Studies Hybridoma Bank by L.B. Knudsen (Developmental Studies Hybridoma Bank Hybridoma Product mAb 3F52) and has previously been validated as specific for the GLP-1R, to map GLP-1R expression.17

Power Calculation and Statistical Analysis
In the coronary blood flow study, patients acted as their own control, and the primary end point for the study was change in BMR. On the basis of previous data, we calculated that 10 paired data sets would provide 80% power to detect a clinically significant difference (ΔBMR, 20 mm Hg.s; SD, 15 mm Hg.s) after administration of GLP-1.

Statistical analyses were performed in GraphPad Prism, Version 6 (GraphPad Software, CA). Continuous data are presented as means (SDs) and were analyzed by paired (serial measures in the same patient) and unpaired (between patient groups) Student t test or Wilcoxon rank, as appropriate. Categorical data are presented as counts or frequencies (percentages) and were analyzed by χ² or Fisher’s exact test, as appropriate. In the forearm blood flow study, forearm blood flow at varying GLP-1 doses was compared using a 1-way repeated-measures ANOVA model. All calculations were 2 tailed, and P<0.05 was deemed statistically significant.

Results
Demographics for patients who received GLP-1 in the forearm and underwent PCI blood flow studies and for control patients in the PCI blood flow study are summarized in Table 1. The groups were well matched in terms of baseline demographics, medical history, and medications. There were significantly more patients with a previous myocardial infarction in the

### Table 2. Coronary Blood Flow Results at Baseline and After GLP-1 or Saline Administration for 20 Minutes

| Variable | Treated Baseline | Treated 20 Minutes After GLP-1 | P Value | Control Baseline | Control 20 Minutes After Stent | P Value |
|----------|-----------------|-------------------------------|--------|-----------------|-------------------------------|--------|
| Heart rate, bpm | 63.7 (7.3) | 63.0 (13.8) | 0.85 | 79.3 (32.1) | 68.7 (11.9) | 0.35 |
| Systolic BP, mm Hg | 136.3 (28.8) | 138.3 (22.3) | 0.59 | 133.1 (36.0) | 140.0 (26.1) | 0.46 |
| Diastolic BP, mm Hg | 59.6 (6.5) | 63.4 (7.7) | 0.08 | 72.3 (17.1) | 71.8 (15.3) | 0.68 |

Nonhyperemic

| Variable | Mean (SD) | Mean (SD) | P Value | Mean (SD) | Mean (SD) | P Value |
|----------|----------|----------|---------|----------|----------|---------|
| Pa, mm Hg | 89.7 (16.5) | 90.8 (16.6) | 0.66 | 97.4 (23.3) | 89.5 (15.3) | 0.13 |
| Pd, mm Hg | 84.6 (16.5) | 84.9 (15.9) | 0.92 | 93.3 (24.1) | 86.3 (16.7) | 0.17 |
| Tmn, s | 0.87 (0.39)* | 0.63 (0.27) | 0.02* | 0.48 (0.24)* | 0.83 (0.41) | <0.001* |
| BMR | 76.3 (37.9) | 55.4 (30.4) | 0.02* | 45.9 (34.7) | 66.7 (37.2) | 0.02* |

Hyperemic

| Variable | Mean (SD) | Mean (SD) | P Value | Mean (SD) | Mean (SD) | P Value |
|----------|----------|----------|---------|----------|----------|---------|
| Pa, mm Hg | 81.2 (17.8) | 80.1 (16.7) | 0.61 | 90.6 (19.9) | 84.9 (18.3) | 0.11 |
| Pd, mm Hg | 71.9 (14.9) | 71.9 (15.3) | 1.00 | 81.6 (19.7) | 76.2 (17.1) | 0.14 |
| Tmn, s | 0.24 (0.10) | 0.29 (0.22) | 0.55 | 0.20 (0.07) | 0.21 (0.07) | 0.87 |
| IMR | 16.3 (10.2) | 19.7 (14.6) | 0.52 | 15.6 (5.8) | 15.0 (6.2) | 0.78 |
| FFR | 0.88 (0.06) | 0.89 (0.06) | 0.49 | 0.89 (0.08) | 0.89 (0.08) | 0.76 |
| CFR | 4.0 (2.2) | 3.0 (2.4) | 0.36 | 2.4 (0.8) | 4.2 (2.0) | <0.01* |
| RRR | 5.0 (2.0) | 3.6 (2.5) | 0.23 | 2.9 (1.3) | 4.7 (2.3) | 0.04* |

Data are given as mean (SD). BMR indicates basal microcirculatory resistance; BP, blood pressure; bpm, beats per minute; CFR, coronary flow reserve; FFR, fractional flow reserve; GLP-1, glucagon-like peptide-1; IMR, index of microcirculatory resistance; Pa, aortic pressure; Pd, distal coronary pressure; RRR, resistance reserve ratio; Tmn, transit time.

*P=0.04 for baseline nonhyperemic Tmn intergroup comparison.
control arm of the PCI study, but other demographic data were similar. In particular, all patients were taking a statin. All patients received drug-eluting stents, and these were a similar size with a similar total ischemic time to the cardiac myocardium, as measured by the total coronary balloon occlusion time.

Figure 5. Comparison of resting transit time (Tmn) and basal microcirculatory resistance (BMR) at baseline and after 20-minute infusion of GLP-1 (glucagon-like peptide-1) (n=10, A and C) or saline control (n=11, B and D) before hyperemia. Both Tmn and BMR decrease after GLP-1, whereas after saline, they both increase, suggesting GLP-1 is a coronary microvascular vasodilator. Changes in resting Tmn (E) and BMR (F) after GLP-1 or saline controls at 20 minutes after percutaneous coronary intervention compared with baseline accentuate this difference.
GLP-1 Coronary Vasodilator

Forearm Blood Flow

Blood pressure and heart rate during the forearm GLP-1 infusions remained constant (Figures 2 and 3). There was no effect of GLP-1 on absolute blood flow in the control (P=0.17) or infused (P=0.77) forearms, forearm blood flow ratio (P=0.19), or percentage change in forearm blood flow ratio (P=0.20) from baseline or any of the 5 incremental doses of GLP-1 (Figure 4). There were no hypoglycemic episodes confirmed by intermittent finger-prick glucometer readings.

Coronary Blood Flow

The changes in mean hemodynamic indexes after PCI at baseline and after 20 minutes of GLP-1 or saline control are summarized in Table 2. GLP-1 had no effect on resting heart rate, systolic or diastolic blood pressure, or mean arterial pressure; there was no change in resting coronary pressure indexes (Table 2). However, GLP-1 did cause a significant reduction in resting Tmn (P=0.02), as exemplified in Figure 1, reflecting an increase in coronary flow velocity that drove down BMR compared with baseline resting values (P=0.02, Figure 5). In saline-infused controls, the Tmn and BMR did the opposite and increased 20 minutes after stenting compared with baseline (P<0.001 and P=0.02 respectively; Table 2 and Figure 5). This reduction in coronary flow velocity increased the CFR and resistance reserve ratio in the control group, although there was no significant influence of saline or GLP-1 on hyperemic pressure or flow velocity indexes. The apparent increase in CFR and resistance reserve ratio after saline was driven by a deterioration basal flow velocity and increase of BMR, leading to an increase in the ratios observed.

When comparing GLP-1 and saline effect between patient groups, the highly significant and opposite difference in resting Tmn response after GLP-1 and saline (P<0.001, Figure 5) was responsible for the difference in change in BMR (P<0.001).

Biochemical data are summarized in Table 3. GLP-1 (7–36) NH₂ and its metabolite (9–36) NH₂ both increased, as expected, after a 20-minute infusion, but GLP-1 had no significant effect on blood glucose, free fatty acid, or insulin concentrations. Simultaneous blood samples taken from the coronary sinus and coronary artery reveal a modest, but significant, transmyocardial extraction of GLP-1 (7–36) (P=0.03) but not GLP-1 (9–36) (P=0.09) (Figure 6).

There was no significant transmyocardial uptake of glucose (−0.13 mmol/L, P=0.1), free fatty acid (−8.9 μmol/L, P=0.26), or insulin (−0.13 pmol/L, P=0.8) during GLP-1 infusion.
Immunohistochemistry

The tissue-specific localization of GLP-1R observed with the mAb 3F52 antibody is summarized in Table 4. As expected, the pancreas had weak staining in acinar cells and high staining in β cells, as illustrated in Figure 7A and 7B, and we confirmed no staining in pancreatic and atrial tissue without the antibody in negative controls (Figures 7C and 7F). Atrial and ventricular tissue both showed moderate staining of GLP-1R in cardiomyocytes, with no apparent localization to any region (Figure 7D, 7E, 7G, and 7H). Coronary microvessels were visible in both atria and ventricle tissue (Figure 7E and 7H), although no staining with the GLP-1R mAb was observed on the vascular endothelium or smooth muscle cells. Figure 8 illustrates that there was also no staining in any vascular specimen examined (aorta, left internal mammary artery, coronary artery, saphenous vein, or radial artery). Two presumably immune cells were visible with moderate staining in the adventitia of the left internal mammary artery section, indicating that the antibody assay had worked (Figure 8E).

Discussion

We have clearly demonstrated that GLP-1 (7–36) NH₂ has no peripheral forearm vasodilatory effect and that the GLP-1R is not expressed in peripheral or coronary vascular endothelium or smooth muscle cells. However, GLP-1 is a coronary microcirculatory vasodilator and is likely to bind to GLP-1Rs on cardiomyocytes, as indicated by transmyocardial GLP-1
(7–36) NH₂ extraction and GLP-1R staining on ventricular cardiomyocytes. There was no significant change in metabolic substrate profile after GLP-1. This suggests that GLP-1 has a direct action on cardiomyocytes and influences coronary microvascular flow and resistance indirectly through ventricular-coronary interactions. We also confirm that coronary flow velocity diminishes with time after PCI in the control group.

GLP-1 agonists have been shown to have beneficial cardiovascular effects in patients with diabetes mellitus.⁷ We have previously shown that GLP-1 can abrogate ischemic left ventricular (LV) dysfunction in a human angioplasty model of stunning.¹²,¹³,³³ Although peripheral hemodynamic effects have been described in animals,¹⁸–²⁰ the ventricular effects of GLP-1 (7–36) are not explained in humans by peripheral vasodilatation and a reduction in afterload. We could not visualize the presence of the GLP-1R in vascular tissue, and forearm blood flow measurements remained constant despite incremental GLP-1 infusion doses. Systemic blood pressure and pulse rate also remained constant in both the coronary and forearm blood flow studies during GLP-1 infusion.

However, we did observe coronary vasodilatation with enhanced coronary flow velocity during GLP-1 (7–36), and this is compatible with the observed augmentation of LV function during ischemia after GLP-1 through a mechanism of ventricular-coronary cross talk. Coronary perfusion and contractility are linked by several factors to match supply and demand. Physical forces from increased inotropy and matched lusitropy are thought to “pull open” the adjacent microvasculature, increasing coronary microcirculatory volume and reducing resistance.³⁴ We have previously shown that the coronary backward expansion wave is proportional to LV diastolic indexes measured by conductance catheter.³⁵ Improved lusitropy also diminishes the compressive forces of end diastolic pressure and wall stress acting on the subendocardial microvasculature from the LV cavity. Microvascular dilatation can, in turn, promote contractility by stretching adjacent ventricular myofibrils and activating stretch-activated calcium channels in cardiomyocytes to augment contraction by the Gregg effect.

Whether GLP-1 acts directly or indirectly on the ventricular myocardium depends on where its receptor is expressed. We confirmed myocardial extraction of GLP-1, and our immunohistochemistry data suggest that GLP-1 may bind to receptors on ventricular myocytes and promote contractility directly, with secondary vasodilatory effects on the coronary microcirculation. However, other groups have shown with immunohistochemistry and gene expression studies that the GLP-1R
Figure 8. Immunohistochemistry sections labelling glucagon-like peptide-1 receptor (GLP-1R) with monoclonal antibody (mAb) 3F52 in human blood vessels. None show positive staining for the GLP-1R. A, Coronary artery, low power (magnification ×20). B, Coronary artery, high power (magnification ×60). C, Aorta, high power (magnification ×60). D, Left internal mammary artery, high power (magnification ×60). E, Adventitia of left internal mammary artery, high power (magnification ×60), which includes 2 presumably immune cells with positive staining for GLP-1R (arrows). F, Saphenous vein, high power (magnification ×60). G, Radial artery, low power (magnification ×20). H, Radial artery, high power (magnification ×60), boxed area of G.
is localized to vascular smooth muscle and sinoatrial tissue rather than ventricular tissue.16,17 If this is the case, our findings could be explained by the Gregg effect. However, a recent study of GLP-1R expression in the human heart showed similar results to those presented herein and identified a low-level expression in all 4 heart chambers, although the expression could not be localized to a specific cell type.36 Furthermore, this study did not detect any GLP-1R expression in coronary artery vascular smooth muscle cells, which corroborate our observations. A metabolic effect of GLP-1 on the heart, switching from free fatty acid use to more energy-efficient glucose metabolism,37 seems unlikely, because we did not observe any changes in plasma substrate availability after GLP-1.

The observation that coronary flow velocity diminishes with time after PCI has been reported before by our group and may be explained by the gradual dissipation of reactive hyperemia and ischemia induced by repeated coronary balloon inflations during PCI.38 The degree of reactive hyperemia is likely to be similar between GLP-1–treated and saline controls because the total ischemic time was similar; although the augmented coronary flow velocity wanes with time in controls, as expected, it is further increased after GLP-1. Indexes may be influenced by changes in basal flow (eg, CFR should be used with caution to gauge the interventional result), because basal flow is not stable after PCI. In addition, the observed increase in CFR and resistance reserve ratio with time after PCI in the control group must also be interpreted with caution; it does not reflect improved hyperemic response in coronary flow velocity, but rather a reduction in resting flow.

Clinical Significance

Our findings are clinically important for patients with type 2 diabetes mellitus. GLP-1–based therapies can be used to improve glycemic control and appear to be associated with either a neutral or even improved cardiovascular profile, unlike some other agents.39 A nonglycemic coronary vasodilatory effect in patients with diabetes mellitus and particularly at risk of morbidity from coronary microvascular dysfunction is desirable. However, we confirm that GLP-1 administration in a nondiabetic population is also safe and may encourage a wider application of this therapy to treat coronary microvascular dysfunction. Our data provide compelling justification to further evaluate the use of GLP-1 in larger studies to improve myocardial blood flow.

Limitations

We did not use a positive control (eg, sodium nitroprusside) in the forearm blood flow experiments to confirm an endothelium-independent vasodilatory effect could be detected with this technique, although this has been shown before.27 We only assessed the direct vasodilatory potential of GLP-1 and not its ability to augment the vasodilatory response of other agents, as has been demonstrated by others.40 Baseline coronary artery flow may be perturbed by external factors, but ambient temperature was constant and noise and discomfort were minimal during data acquisition. We accept that basal flow velocity is variable, and the response to GLP-1 will depend on basal coronary flow. An effect of GLP-1 may only be observed in individuals with abnormal basal flow, as is frequently encountered in patients with diabetes mellitus, although confirmation of our findings in this patient group is warranted. The subtle GLP-1 coronary vasodilatory effect can be swamped by exogenous vasodilators (eg, adenosine). Hyperemic indexes are usually used clinically to determine coronary microvascular integrity to overcome limitations of basal flow variability; however, they are artificial, pharmacologically induced readings that are not steady state and, as we have observed, may be misleading if basal flow conditions are ignored.

We did not assess the LV function before recruitment, and we did not assess it intraprocedurally, to confirm our previous findings of abrogation of ischemic LV dysfunction and stunning after GLP-1. This was also not a randomized controlled trial, but rather patients acted as their own control, and as such, investigators were not blinded to treatment allocation.

This was a human study and, therefore, our physiological and histopathological data could not be gathered from the same subject. However, the myocardial extraction data, paired with our histopathological data confirming GLP-1R presence on ventricular tissue, are persuasive. Although we used a well-characterized mAb 3F52 specific to the GLP-1R, we did not confirm the presence of the GLP-1R with another method, and it is possible an alternative tissue binding pattern and mechanism may be responsible for our observations. For example, GLP-1 and its metabolite have been demonstrated to act independently of the GLP-1R in a rodent model,41 and this may also be the case in humans. Finally, we also did not definitively confirm GLP-1 binding; degradation could also explain the transmyocardial gradient we observed.

Conclusion

GLP-1 causes coronary microvascular dilation but does not influence peripheral vascular tone. GLP-1Rs are expressed on ventricular cardiomyocytes but not vascular tissue, and transmyocardial GLP-1 extraction occurs, suggesting that the GLP-1 effect on the coronary microvasculature is indirectly mediated by ventricular-coronary cross talk.
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Disclosures
Clarke is employed by MSD. This work has been performed independently. Cheriyan is employed by Cambridge University Hospitals National Health Service Foundation Trust and is obliged to spend 50% of his time on GlaxoSmithKline-related clinical trial work but receives no employee benefits from this relationship. The remaining authors have no disclosures to report.

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