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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 bloods 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. (*J Am Heart Assoc*. 2018;7:e010321. DOI: 10.1161/JAHA.118.010321.)

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

Ischemic heart disease resulting from coronary artery disease remains the leading cause of death globally. Type 2 diabetes mellitus is a significant cardiovascular risk factor

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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

Clinical Perspective

What Is New?

- We demonstrate that the incretin hormone GLP-1 (glucagon-like peptide 1) does not have an effect on peripheral vasculature tone in humans but is a coronary vasodilator and reduces coronary microcirculatory resistance.
- GLP-1 myocardial extraction is observed, but GLP-1 receptor expression is not found, on human vasculature, although it is expressed on left ventricular tissue.
- GLP-1 does not appear to significantly alter the availability of myocardial substrate.

What Are the Clinical Implications?

- GLP-1 may exert its cardioprotective effect, which is independent of glycemic control, by reducing coronary microcirculatory resistance and improving myocardial blood flow.

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 cells[16,17]; and active GLP-1 (7–36) NH_2 and its metabolite (9–36) NH_2 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; Hameln 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
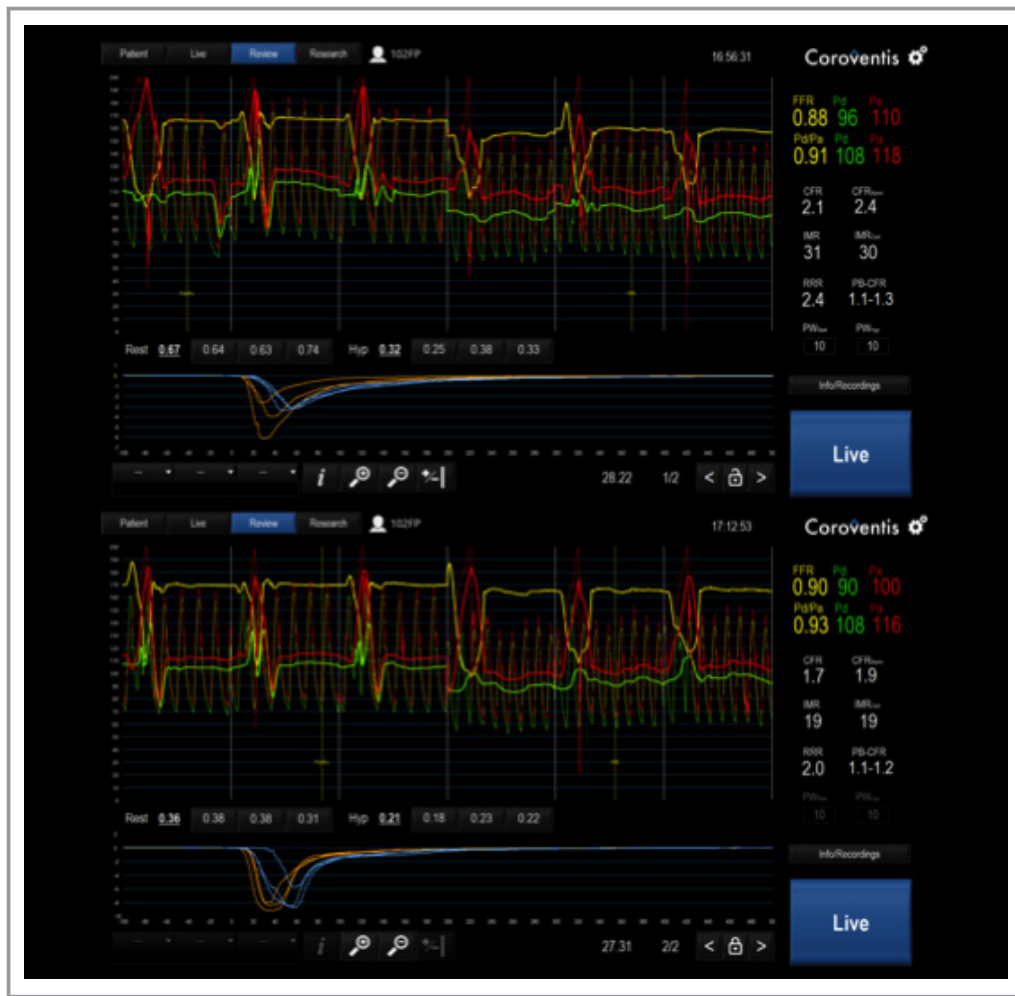
Figure 1. Representative screen shots of pressure wire–derived indexes measured in the same patient at baseline (top panel) after percutaneous coronary intervention and after a 20-minute infusion of GLP-1 (glucagonlike peptide-1; bottom panel). After GLP-1, the mean resting transit time (T_{mn}; blue thermodilution curves) shortens from 0.67 to 0.36 seconds because of increased resting flow velocity, and this reduces calculated basal microcirculatory resistance (BMR). CFR indicates coronary flow reserve; FFR, fractional flow reserve; IMR, index of microcirculatory resistance; P_a, aortic pressure; P_d, coronary distal pressure.

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 (P_v) and blood sampling from the coronary sinus. The coronary guide catheter provided access for coronary artery blood sampling.

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/m^2	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.

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 (T_{mn}) of an intracoronary injectate of room-temperature saline (thermodilution technique), as previously described.[28–30] Coronary microcirculatory hyperemia was induced by intravenous administration of adenosine, 0.14 mg/kg per minute, into the femoral vein. At maximal hyperemia, T_{mn}, aortic pressure (P_a), and coronary distal pressure (P_d) were recorded (Figure 1). Coronary wedge pressure (P_w) was measured separately as P_d 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 P_d as necessary.
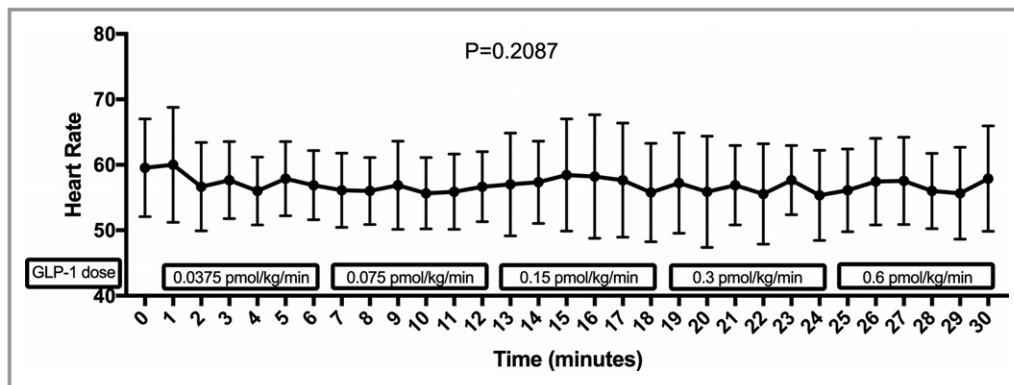

Figure 2. No significant change in heart rate from baseline and every minute during the 6 infused doses of GLP-1 (glucagon-like peptide-1 [7–36]): 0.0375, 0.075, 0.15, 0.3, and 0.6 pmol/kg per minute; n=10.
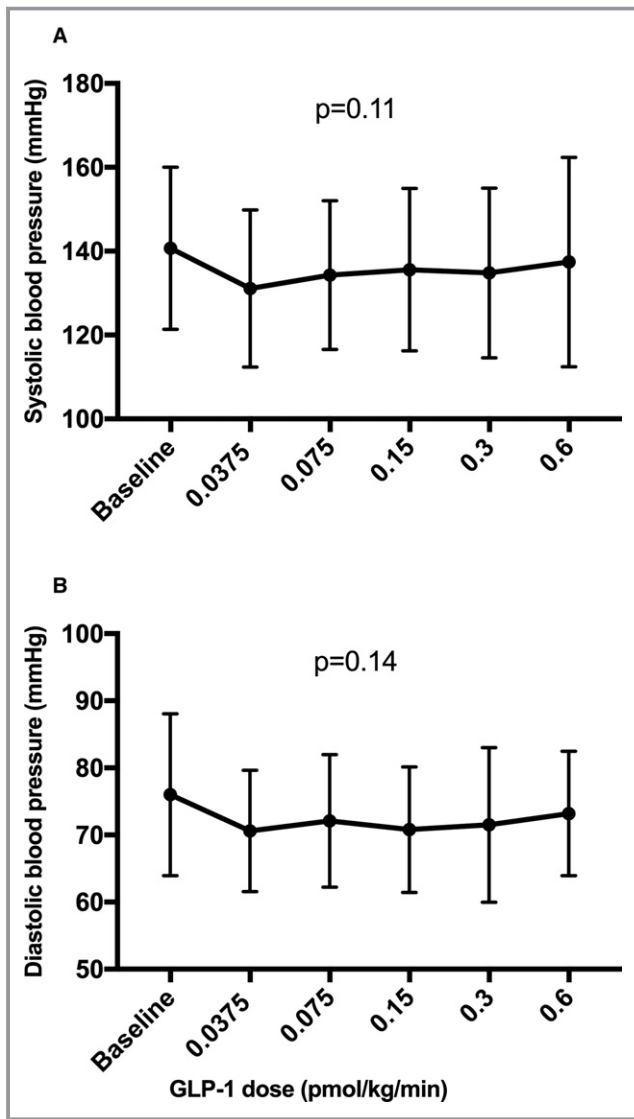
Figure 3. No change from baseline in systolic (A) or diastolic (B) blood pressure during the 6 infused doses of glucagon-like peptide-1 (GLP-1 [7–36]) over 30 minutes; n=10.

Figure 4. A, Absolute change in forearm blood flow did not change significantly in either the control (*P*=0.17) or infused (*P*=0.77) arms. B, Percentage change in forearm blood flow ratio between the control and infused arms was also not different (*P*=0.20); n=10. GLP-1 indicates glucagon-like peptide-1.

These measurements enabled the calculation of the following: index of microcirculatory resistance (mm Hg/s or units)=$P_a \times T_{mn} \times [(P_d-P_w)/(P_a-P_w)]_{hyperemia}$ corrected for collaterals[12]; basal microcirculatory resistance (BMR; mm Hg/s or units)=$P_a \times T_{mn} \times [(P_d-P_w)/(P_a-P_w)]_{baseline}$; resistance reserve ratio=BMR/index of microcirculatory resistance; coronary flow reserve (CFR)=$T_{mn(baseline)}/T_{mn(hyperemia)}$; and fractional flow reserve=$(P_d-P_v)/(P_a-P_v)_{hyperemia}$.

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.
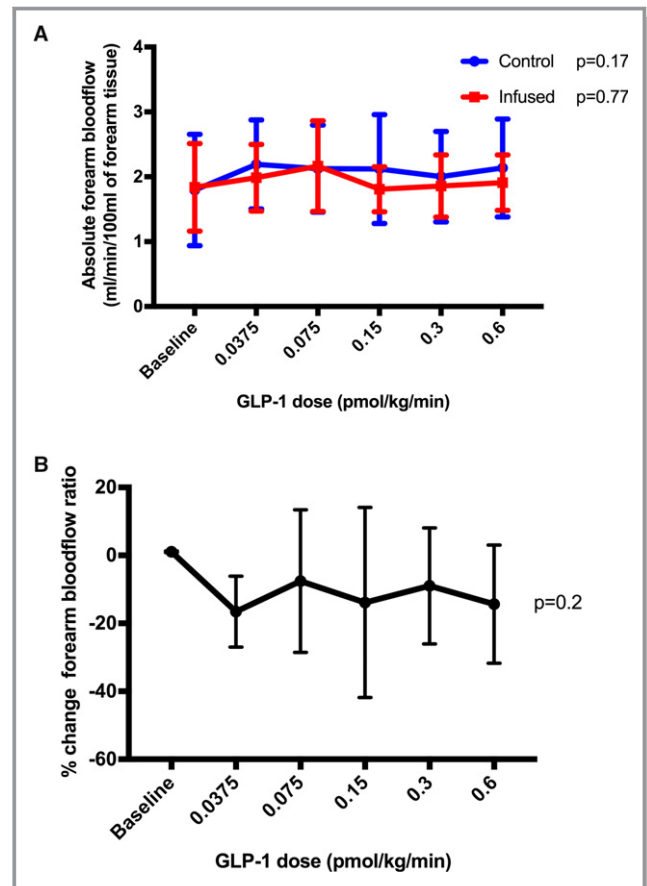
Biochemistry

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) NH$_2$ and the GLP-1 metabolite (9–36) NH$_2$ 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.

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						
P_a, mm Hg	89.7 (16.5)	90.8 (16.6)	0.66	97.4 (23.3)	89.5 (15.3)	0.13
P_d, mm Hg	84.6 (16.5)	84.9 (15.9)	0.92	93.3 (24.1)	86.3 (16.7)	0.17
T_{mn}, 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						
P_a, mm Hg	81.2 (17.8)	80.1 (16.7)	0.61	90.6 (19.9)	84.9 (18.3)	0.11
P_d, mm Hg	71.9 (14.9)	71.9 (15.3)	1.00	81.6 (19.7)	76.2 (17.1)	0.14
T_{mn}, 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; P_a, aortic pressure; P_d, distal coronary pressure; RRR, resistance reserve ratio; T_{mn}, transit time.
*P=0.04 for baseline nonhyperemic T_{mn} intergroup comparison.

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 χ^2 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
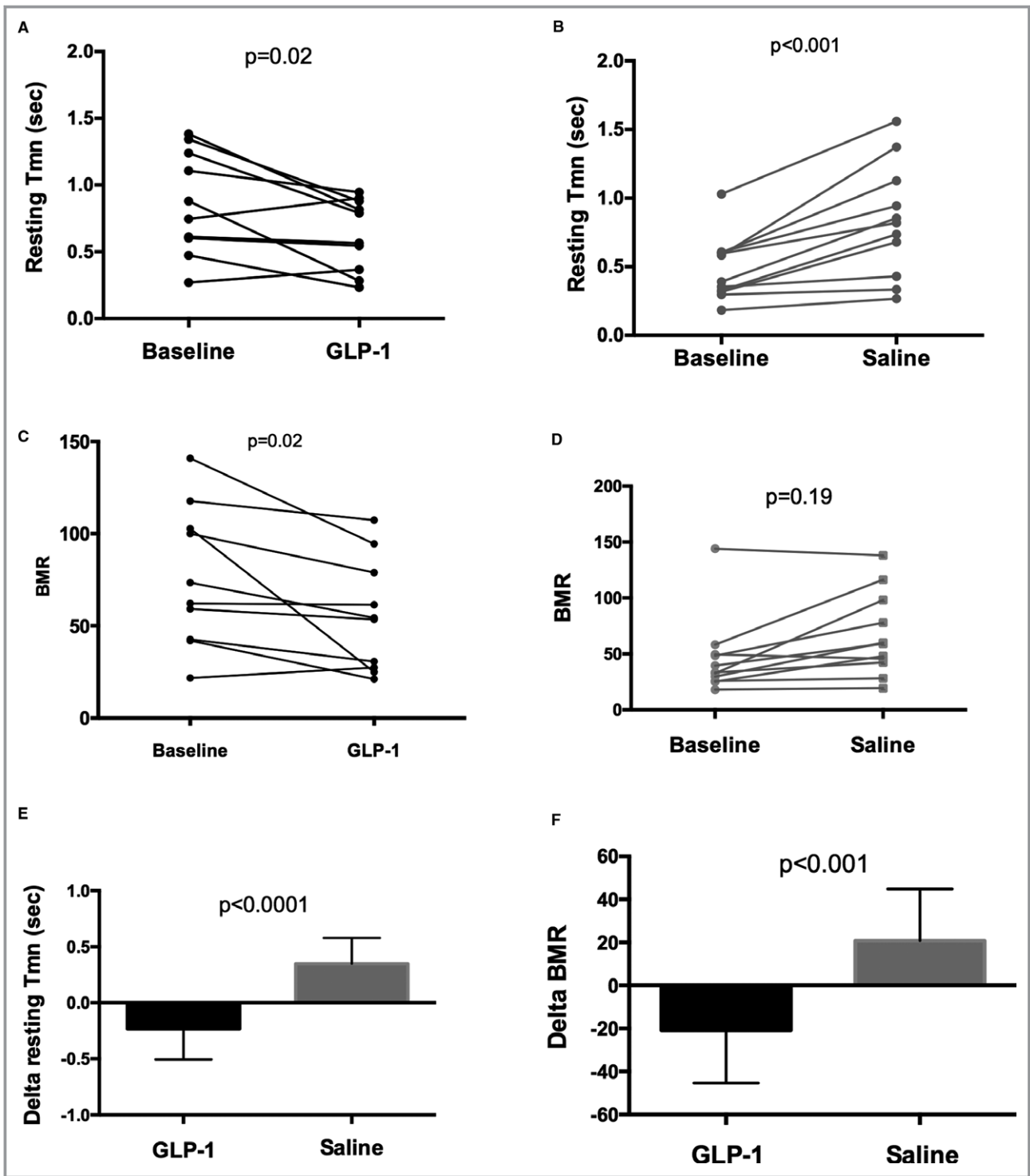
Figure 5. Comparison of resting transit time (T$_{mn}$) and basal microcirculatory resistance (BMR) at baseline and after 20-minute infusion of GLP-1 (glucagon-like peptide-1) (n=10, A and C) or saline control (n=11, B and D) before hyperemia. Both T$_{mn}$ and BMR decrease after GLP-1, whereas after saline, they both increase, suggesting GLP-1 is a coronary microvascular vasodilator. Changes in resting T$_{mn}$ (E) and BMR (F) after GLP-1 or saline controls at 20 minutes after percutaneous coronary intervention compared with baseline accentuate this difference.

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.

Table 3. Peripheral Venous Plasma Biochemistry Results at Baseline and After GLP-1 Administration for 20 Minutes

Variable	Baseline	GLP-1	P Value
Blood glucose, mmol/L	6.4 (1.7)	5.9 (1.2)	0.15
Free fatty acid, μmol/L	1326 (600.5)	1157 (600.5)	0.14
Insulin, pmol/L	68.44 (68.4)	60.7 (47.6)	0.20
GLP-1 (7–36), pmol/L	1.3 (1.9)	16.2 (7.7)	<0.001
GLP-1 (9–36), mol/L	6.8 (3.2)	46.7 (14.4)	<0.0001

Data are given as mean (SD). GLP-1 indicates glucagon-like peptide-1.

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
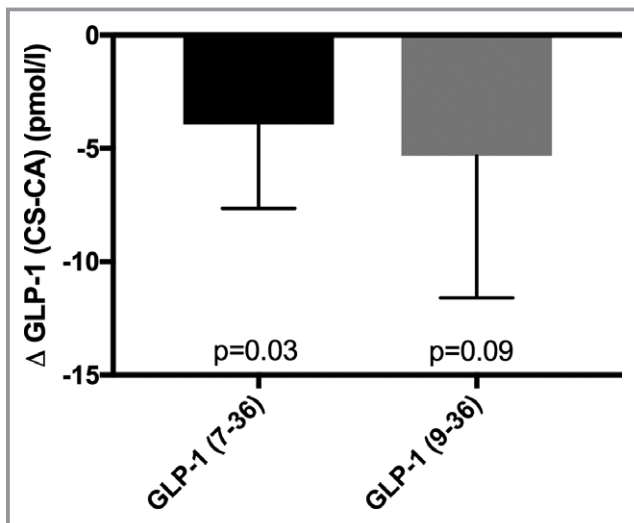

Figure 6. Transmyocardial extraction of GLP-1 (glucagon-like peptide-1 [7–36] and GLP-1 [9–36]) calculated by simultaneously collected blood sample analysis from coronary artery (CA) and coronary sinus (CS); n=10.

Table 4. GLP-1 Localization by Tissue Type Using Immunohistochemistry With mAb 3F52

Tissue	GLP-1R–Expressing Cells	Intensity of GLP-1R Staining
Pancreas	β cells Acinar cells	+++ +
Atria	Cardiomyocytes	++
Ventricle	Cardiomyocytes	++
Aorta	No staining	
Coronary artery	No staining	
Radial artery	No staining	
Left internal mammary artery	No staining	
Saphenous vein	No staining	

GLP-1 indicates glucagon-like peptide-1; GLP-1R, GLP-1 receptor; mAb, monoclonal antibody; +, low; ++, medium; +++, high.

indexes (Table 2). However, GLP-1 did cause a significant reduction in resting T_{mn} ($P=0.02$), 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 T_{mn} 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 T_{mn} response after GLP-1 and saline ($P<0.001$, Figure 5) was responsible for the difference in change in BMR ($P<0.001$).

Biochemistry

Biochemical data are summarized in Table 3. GLP-1 (7–36) NH_2 and its metabolite (9–36) NH_2 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.
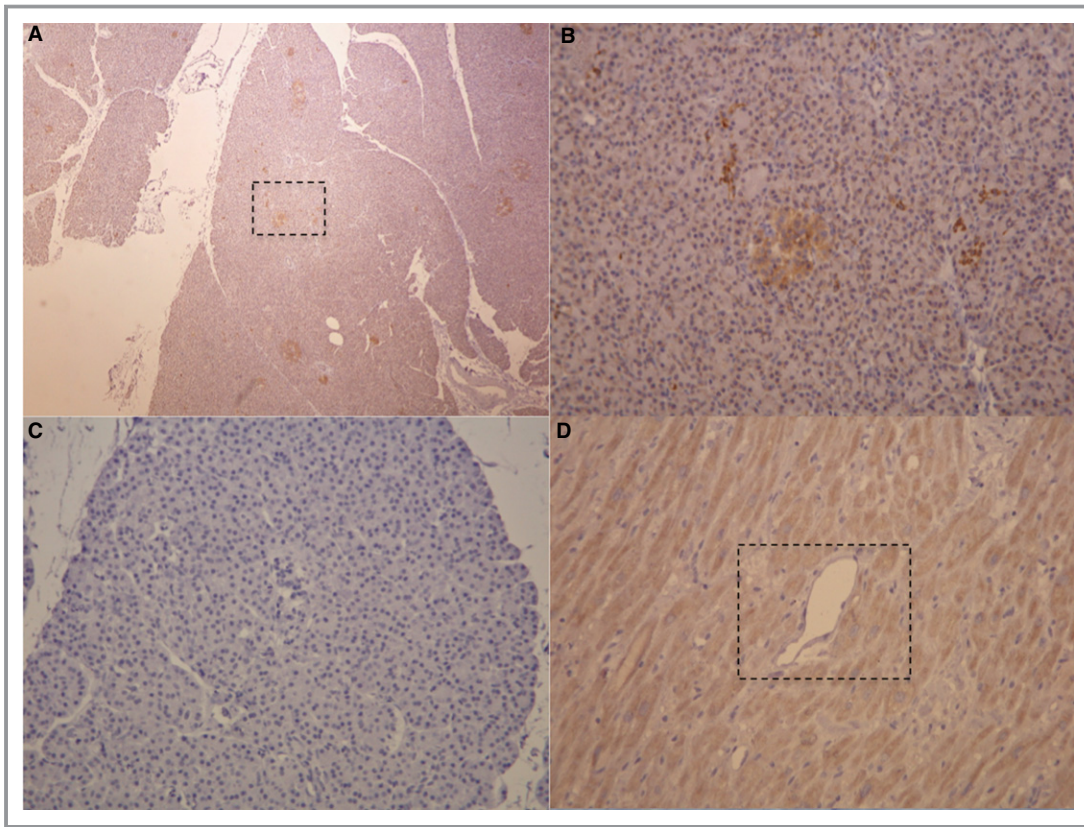
Figure 7. Immunohistochemistry sections labelling the glucagon-like peptide-1 receptor (GLP-1R) with and without monoclonal antibody (mAb) 3F52. A, Pancreatic tissue, low power (magnification ×20), positive control with mAb 3F52, showing moderate widespread staining for GLP-1R and high staining for GLP-1R in β cells. B, Pancreatic tissue, positive control with mAb 3F52, high power (magnification ×60), boxed area of A. C, Pancreatic tissue, negative control without mAb 3F52, high power (magnification ×60), confirming no staining. D, Atrial tissue with mAb 3F52, low power (magnification ×20), showing moderate widespread staining for GLP-1R in atrial cardiomyocytes. E, Atrial tissue, with mAb 3F52, high power (magnification ×60), boxed area of D, showing staining of cardiomyocytes but no staining in the endothelial cells of a coronary vessel (arrow). F, Atrial tissue, negative control without mAb 3F52, high power (magnification ×60), without staining. G, Ventricular tissue with mAb 3F52, low power (magnification ×20), showing moderate widespread staining for GLP-1R in ventricular cardiomyocytes. H, Ventricular tissue with mAb 3F52, high power (magnification ×60), boxed area of G, showing moderate widespread staining for GLP-1R in cardiomyocytes but no staining on the endothelial cells of a coronary vessel (arrow).

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) NH_2 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
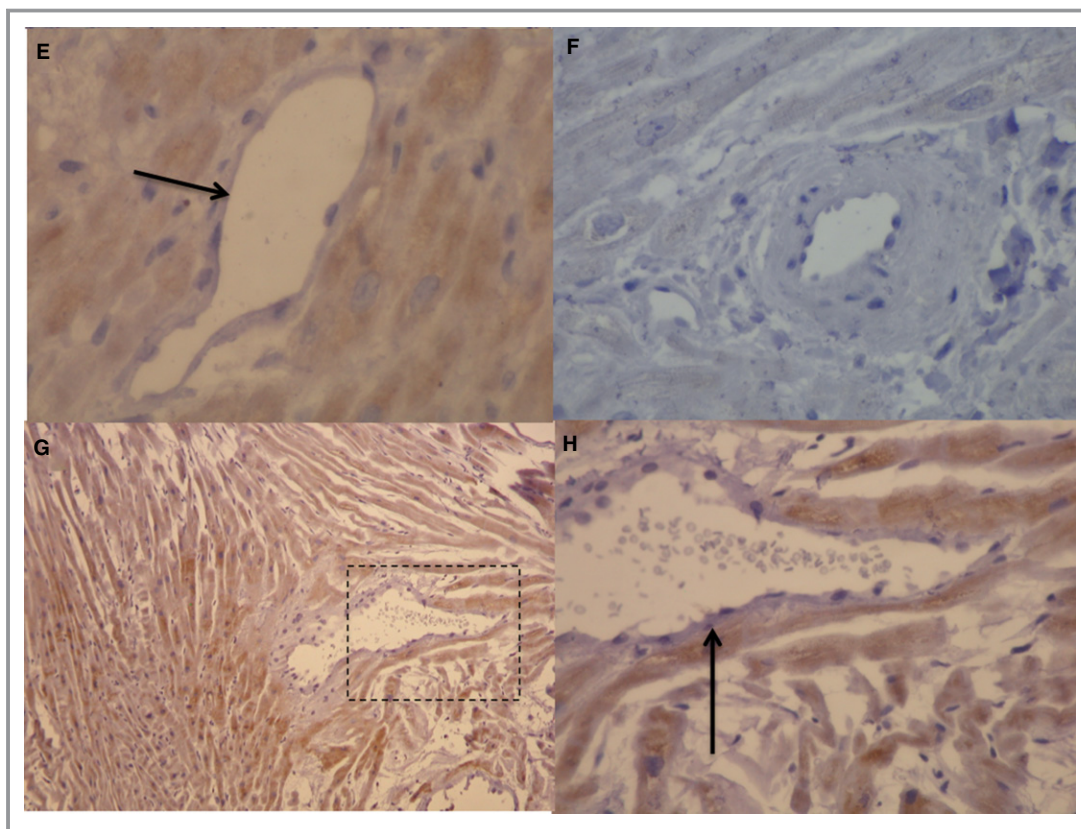
Figure 7. Continued.

(7–36) NH$_2$ 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
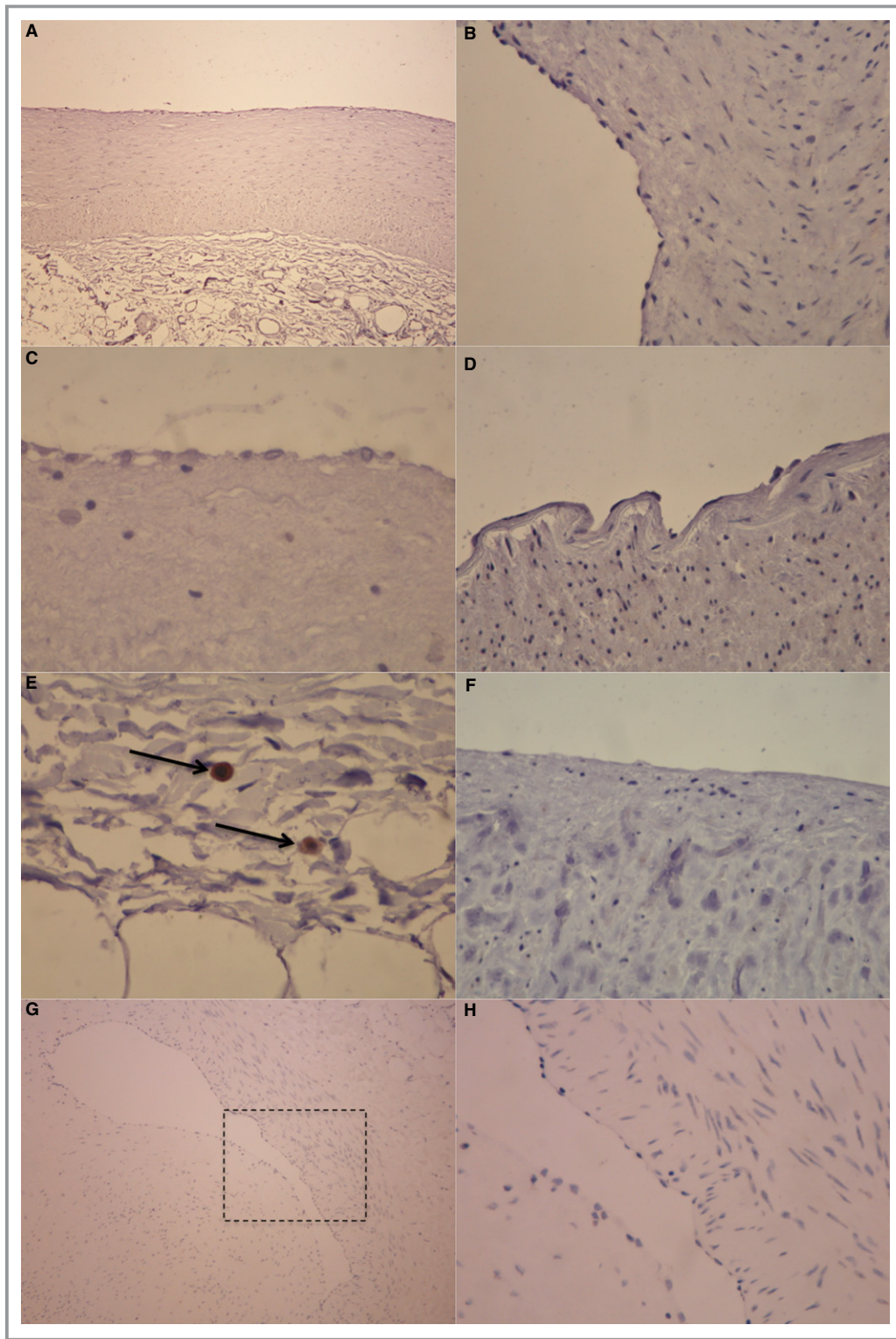
Figure 8. Immunohistochemistry sections labelling glucagon-like peptide-1 receptor (GLP-1R) with monoclonal antibody (mAb) 3F52 in human blood vessels. None show positive staining for the GLP-1R. A, Coronary artery, low power (magnification ×20). B, Coronary artery, high power (magnification ×60). C, 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 also 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.

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 obligated 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.

References

1. Nagendran J, Oudit GY, Bakal JA, Light PE, Dyck JR, McAlister FA. Are users of sulphonylureas at the time of an acute coronary syndrome at risk of poorer outcomes? *Diabetes Obes Metab*. 2013;15:1022–1028.

2. Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med*. 1998;339:229–234.

3. McMurray JJ, Packer M, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, Rouleau JL, Shi VC, Solomon SD, Swedberg K, Zile MR; PARADIGM-HF Investigators and Committees. Angiotensin-neprilysin inhibition versus enalapril in heart failure. *N Engl J Med*. 2014;371:993–1004.

4. Wolsk E, Claggett B, Kober L, Pocock S, Yusuf S, Swedberg K, McMurray JJV, Granger CB, Pfeffer MA, Solomon SD. Contribution of cardiac and extra-cardiac disease burden to risk of cardiovascular outcomes varies by ejection fraction in heart failure. *Eur J Heart Fail*. 2017;20:504–510.

5. Lago RM, Nesto RW. Type 2 diabetes and coronary heart disease: focus on myocardial infarction. *Curr DiabRep*. 2009;9:73–78.

6. Folli F, Corradi D, Fanti P, Davalli A, Paez A, Giaccari A, Perego C, Muscogiuri G. The role of oxidative stress in the pathogenesis of type 2 diabetes mellitus micro- and macrovascular complications: avenues for a mechanistic-based therapeutic approach. *Curr Diabetes Rev*. 2011;7:313–324.

7. Marso SP, Daniels GH, Brown-Frandsen K, Kristensen P, Mann JF, Nauck MA, Nissen SE, Pocock S, Poulter NR, Ravn LS, Steinberg WM, Stockner M, Zinman B, Bergenstal RM, Buse JB; LEADER Steering Committee, LEADER Trial Investigators. Liraglutide and cardiovascular outcomes in type 2 diabetes. *N Engl J Med*. 2016;375:311–322.

8. Holman RR, Peterson ED. Sitagliptin and cardiovascular outcomes in type 2 diabetes. *N Engl J Med*. 2015;373:2479.

9. Holman RR, Bethel MA, Mentz RJ, Thompson VP, Lokhnygina Y, Buse JB, Chan JC, Choi J, Gustavson SM, Iqbal N, Maggioni AP, Marso SP, Ohman P, Pagidipati NJ, Poulter N, Ramachandran A, Zinman B, Hernandez AF; EXSCEL Study Group. Effects of once-weekly exenatide on cardiovascular outcomes in type 2 diabetes. *N Engl J Med*. 2017;377:1228–1239.

10. Lonborg J, Vejlstrup N, Kelbaek H, Botker HE, Kim WY, Mathiasen AB, Jorgensen E, Helqvist S, Saunamaki K, Clemmensen P, Holmvang L, Thuesen L, Krusell LR, Jensen JS, Kober L, Treiman M, Holst JJ, Engstrom T. Exenatide reduces reperfusion injury in patients with ST-segment elevation myocardial infarction. *Eur Heart J*. 2012;33:1491–1499.

11. Lonborg J, Kelbaek H, Vejlstrup N, Botker HE, Kim WY, Holmvang L, Jorgensen E, Helqvist S, Saunamaki K, Terkelsen CJ, Schoos MM, Kober L, Clemmensen P, Treiman M, Engstrom T. Exenatide reduces final infarct size in patients with ST-segment-elevation myocardial infarction and short-duration of ischemia. *Circ Cardiovasc Interv*. 2012;5:288–295.

12. McCormick LM, Hoole SP, White PA, Read PA, Axell RG, Clarke SJ, O'Sullivan M, West NE, Dutka DP. Pre-treatment with glucagon-like peptide-1 protects against ischemic left ventricular dysfunction and stunning without a detected difference in myocardial substrate utilization. *JACC Cardiovasc Interv*. 2015;8:292–301.

13. Read PA, Hoole SP, White PA, Khan FZ, O'Sullivan M, West NE, Dutka DP. A pilot study to assess whether glucagon-like peptide-1 protects the heart from ischemic dysfunction and attenuates stunning after coronary balloon occlusion in humans. *Circ Cardiovasc Interv*. 2011;4:266–272.

14. Read PA, Khan FZ, Heck PM, Hoole SP, Dutka DP. DPP-4 inhibition by sitagliptin improves the myocardial response to dobutamine stress and mitigates stunning in a pilot study of patients with coronary artery disease. *Circ Cardiovasc Imaging*. 2010;3:195–201.

15. Marso SP, Bain SC, Consoli A, Eliaschewitz FG, Jodar E, Leiter LA, Lingvay I, Rosenstock J, Seufert J, Warren ML, Woo V, Hansen O, Holst AG, Pettersson J, Vilsboll T; SUSTAIN-6 Investigators. Semaglutide and cardiovascular outcomes in patients with type 2 diabetes. *N Engl J Med*. 2016;375:1834–1844.

16. Richards P, Parker HE, Adriaenssens AE, Hodgson JM, Cork SC, Trapp S, Gribble FM, Reimann F. Identification and characterization of GLP-1 receptor-expressing cells using a new transgenic mouse model. *Diabetes*. 2014;63:1224–1233.

17. Pyke C, Heller RS, Kirk RK, Orskov C, Reedtz-Runge S, Kaastrup P, Hvelplund A, Bardram L, Calatayud D, Knudsen LB. GLP-1 receptor localization in monkey and human tissue: novel distribution revealed with extensively validated monoclonal antibody. *Endocrinology*. 2014;155:1280–1290.

18. Golpon HA, Puechner A, Welte T, Wichert PV, Feddersen CO. Vasorelaxant effect of glucagon-like peptide-(7-36)amide and amylin on the pulmonary circulation of the rat. *Regul Pept*. 2001;102:81–86.

19. Green BD, Hand KV, Dougan JE, McDonnell BM, Cassidy RS, Grieve DJ. GLP-1 and related peptides cause concentration-dependent relaxation of rat aorta through a pathway involving KATP and cAMP. *Arch Biochem Biophys*. 2008;478:136–142.

20. Nystrom T, Gonon AT, Sjoholm A, Pernow J. Glucagon-like peptide-1 relaxes rat conduit arteries via an endothelium-independent mechanism. *Regul Pept*. 2005;125:173–177.

21. Nystrom T, Gutniak MK, Zhang Q, Zhang F, Holst JJ, Ahren B, Sjoholm A. Effects of glucagon-like peptide-1 on endothelial function in type 2 diabetes patients with stable coronary artery disease. *Am J Physiol Endocrinol Metab*. 2004;287:E1209–E1215.

22. Subaran SC, Sauder MA, Chai W, Jahn LA, Fowler DE, Aylor KW, Basu A, Liu Z. GLP-1 at physiological concentrations recruits skeletal and cardiac muscle microvasculature in healthy humans. *Clin Sci*. 2014;127:163–170.

23. Asmar A, Asmar M, Simonsen L, Madsbad S, Holst JJ, Hartmann B, Sorensen CM, Bulow J. Glucagon-like peptide-1 elicits vasodilation in adipose tissue and skeletal muscle in healthy men. *Physiol Rep*. 2017;5:e13073.

24. Ceriello A, Novials A, Canivell S, La Sala L, Pujadas G, Esposito K, Testa R, Bucciarelli L, Rondinelli M, Genovese S. Simultaneous GLP-1 and insulin administration acutely enhances their vasodilatory, antiinflammatory, and antioxidant action in type 2 diabetes. *Diabetes Care*. 2014;37:1938–1943.

25. Mendis B, Simpson E, MacDonald I, Mansell P. Investigation of the haemodynamic effects of exenatide in healthy male subjects. *Br J Clin Pharmacol*. 2012;74:437–444.

26. Wilkinson IB, Webb DJ. Venous occlusion plethysmography in cardiovascular research: methodology and clinical applications. *Br J Clin Pharmacol*. 2001;52:631–646.

27. Cheriyan J, Webb AJ, Sarov-Blat L, Elkhawad M, Wallace SM, Maki-Petaja KM, Collier DJ, Morgan J, Fang Z, Willette RN, Lepore JJ, Cockcroft JR, Sprecher DL, Wilkinson IB. Inhibition of p38 mitogen-activated protein kinase improves nitric oxide–mediated vasodilatation and reduces inflammation in hypercholesterolemia. *Circulation*. 2011;123:515–523.

28. Pijls NH, De Bruyne B, Smith L, Aarnoudse W, Barbato E, Bartunek J, Bech GJ, Van De Vosse F. Coronary thermodilution to assess flow reserve: validation in humans. *Circulation*. 2002;105:2482–2486.

29. Fearon WF, Farouque HM, Balsam LB, Caffarelli AD, Cooke DT, Robbins RC, Fitzgerald PJ, Yeung AC, Yock PG. Comparison of coronary thermodilution and

Doppler velocity for assessing coronary flow reserve. *Circulation*. 2003;108:2198–2200.

30. De Bruyne B, Pijls NH, Smith L, Wievegg M, Heyndrickx GR. Coronary thermodilution to assess flow reserve: experimental validation. *Circulation*. 2001;104:2003–2006.

31. Wewer Albrechtsen NJ, Bak MJ, Hartmann B, Christensen LW, Kuhre RE, Deacon CF, Holst JJ. Stability of glucagon-like peptide 1 and glucagon in human plasma. *Endocr Connect*. 2015;4:50–57.

32. Wewer Albrechtsen NJ, Asmar A, Jensen F, Torang S, Simonsen L, Kuhre RE, Asmar M, Veedfald S, Plamboeck A, Knop FK, Vilsboll T, Madsbad S, Nauck MA, Deacon CF, Bulow J, Holst JJ, Hartmann B. A sandwich ELISA for measurement of the primary glucagon-like peptide-1 metabolite. *Am J Physiol Endocrinol Metab*. 2017;313:E284–E291.

33. McCormick LM, Heck PM, Ring LS, Kydd AC, Clarke SJ, Hoole SP, Dutka DP. Glucagon-like peptide-1 protects against ischemic left ventricular dysfunction during hyperglycemia in patients with coronary artery disease and type 2 diabetes mellitus. *Cardiovasc Diabetol*. 2015;14:102.

34. Westerhof N, Boer C, Lamberts RR, Sipkema P. Cross-talk between cardiac muscle and coronary vasculature. *Physiol Rev*. 2006;86:1263–1308.

35. Ladwiniec A, White PA, Nijjer SS, O'Sullivan M, West NE, Davies JE, Hoole SP. Diastolic backward-traveling decompression (suction) wave correlates with simultaneously acquired indices of diastolic function and is reduced in left ventricular stunning. *Circ Cardiovasc Interv*. 2016;9:e003779.

36. Baggio LL, Yusta B, Mulvihill EE, Cao X, Streutker CJ, Butany J, Cappola TP, Margulies KB, Drucker DJ. GLP-1 receptor expression within the human heart. *Endocrinology*. 2018;159:1570–1584.

37. Depre C, Vanoverschelde JLJ, Taegtmeyer H. Glucose for the heart. *Circulation*. 1999;99:578–588.

38. Hoole SP, Heck PM, White PA, Read PA, Khan SN, West NE, O'Sullivan M, Dutka DP. Stunning and cumulative left ventricular dysfunction occurs late after coronary balloon occlusion in humans insights from simultaneous coronary and left ventricular hemodynamic assessment. *JACC Cardiovasc Interv*. 2010;3:412–418.

39. Bethel MA, Patel RA, Merrill P, Lokhnygina Y, Buse JB, Mentz RJ, Pagidipati NJ, Chan JC, Gustavson SM, Iqbal N, Maggioni AP, Ohman P, Poulter NR, Ramachandran A, Zinman B, Hernandez AF, Holman RR; EXSCEL Study Group. Cardiovascular outcomes with glucagon-like peptide-1 receptor agonists in patients with type 2 diabetes: a meta-analysis. *Lancet Diabetes Endocrinol*. 2018;6:105–113.

40. Basu A, Charkoudian N, Schrage W, Rizza RA, Basu R, Joyner MJ. Beneficial effects of GLP-1 on endothelial function in humans: dampening by glyburide but not by glimepiride. *Am J Physiol Endocrinol Metab*. 2007;293:E1289–E1295.

41. Ban K, Noyan-Ashraf MH, Hoefer J, Bolz SS, Drucker DJ, Husain M. Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. *Circulation*. 2008;117:2340–2350.