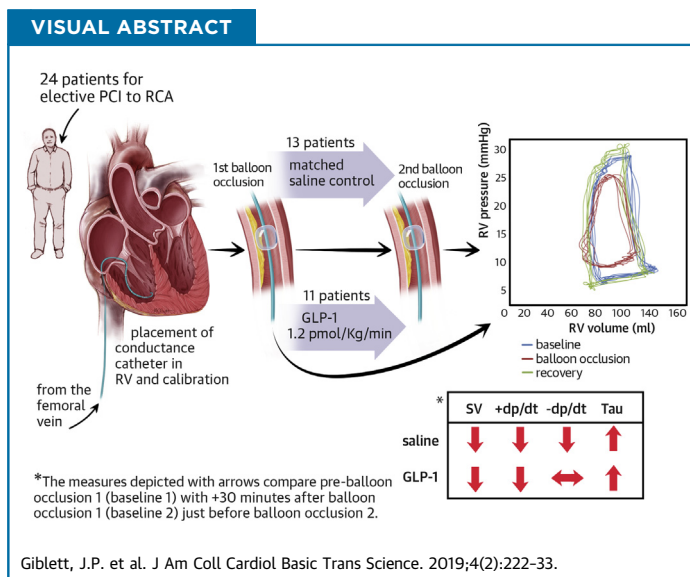


PRECLINICAL RESEARCH

# Glucagon-Like Peptide-1-Mediated Cardioprotection Does Not Reduce Right Ventricular Stunning and Cumulative Ischemic Dysfunction After Coronary Balloon Occlusion



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**HIGHLIGHTS**

- GLP-1 protects against ischemic left ventricular dysfunction after serial coronary balloon occlusion of the left anterior descending artery
- This study assessed whether serial right coronary artery balloon occlusion affected the right ventricle in a similar fashion using a conductance catheter method
- Serial balloon occlusion of the right coronary artery causes stunning and cumulative ischemic dysfunction in the right ventricle
- GLP-1 did not protect against stunning and cumulative ischemic dysfunction in the right ventricle

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All authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Basic to Translational Science* [author instructions page](#).

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## SUMMARY

Stunning and cumulative ischemic dysfunction occur in the left ventricle with coronary balloon occlusion. Glucagon-like peptide (GLP)-1 protects the left ventricle against this dysfunction. This study used a conductance catheter method to evaluate whether the right ventricle (RV) developed similar dysfunction during right coronary artery balloon occlusion and whether GLP-1 was protective. In this study, the RV underwent significant stunning and cumulative ischemic dysfunction with right coronary artery balloon occlusion. However, GLP-1 did not protect the RV against this dysfunction when infused after balloon occlusion. (J Am Coll Cardiol Basic Trans Science 2019;4:222-33) © 2019 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

The importance of the right ventricle in the pathophysiology of heart disease is of increasing clinical relevance (1). Involvement of the right ventricle in myocardial infarction raises the risk of cardiogenic shock and increases mortality, even when treated with primary percutaneous coronary intervention (PCI) (2). Pre-existing right ventricular (RV) failure portends poor prognosis in several conditions (3), and acute deterioration in RV function often has important hemodynamic and clinical consequences.

The blood supply to the right ventricle depends on the coronary anatomy. In a right-dominant system (80%), the right coronary artery (RCA) supplies most of the right ventricle (4). The right ventricle is believed to be relatively resistant to ischemia compared with the left ventricle, as propelling blood into a low-resistance pulmonary circulation requires less work. The right ventricle has thinner, less muscular walls with a lower energetic demand and a lower nutrient/oxygen requirement as a result (5). Coronary balloon inflation during PCI provides a model of supply ischemia. Brief coronary balloon occlusion of the RCA reduces RV stroke volume and stroke work, while there is persistent deterioration of both systolic and diastolic function at 15 min after reperfusion (6,7). Studies of brief coronary occlusion on left ventricular (LV) function suggest that, after transient improvement resulting from reactive hyperemia, residual ventricular dysfunction is revealed (stunning) when coronary flow normalizes at some point after reperfusion (8).

Glucagon-like peptide (GLP)-1 is an incretin hormone, produced from L cells in response to food bolus. GLP-1 receptor (GLP-1R) agonists such as exenatide and liraglutide are used in the management of diabetes mellitus. Data from large trials have shown that these agents have cardiovascular benefits (9,10). Native GLP-1 has been shown to protect against stunning and cumulative ischemic dysfunction in the

left ventricle, whether administered before or after balloon occlusion (11-13).

Animal studies have found that GLP-1 protection against lethal ischemia-reperfusion in the left ventricle is dependent on intracellular signaling pathways involving p70s6K and the phosphoinositol-3-kinase-Akt complex (14-16). These signal cascades are important in the transduction of ischemic preconditioning, and the final effector is the mitochondrial potassium-adenosine triphosphate channel (m-KATP channel). However, blockade of the m-KATP channel, a final effector of ischemic preconditioning, did not abrogate GLP-1 protection in humans (13). Similarly, animal models have implicated changes in myocardial metabolism in GLP-1 cardioprotection (17-21), but a series of human studies have cast doubt on altered substrate use as the cause (12,22,23). A recent study found that GLP-1 is a coronary-specific vasodilator in humans but does not exert its ventricular effect by reducing systemic vascular tone. This study also confirmed that the GLP-1R was present on LV cardiomyocytes but was not expressed on vascular tissue, and thus GLP-1 is likely to have a direct ventricular effect, with secondary vasodilator effects mediated by ventricular-arterial cross-talk (24).

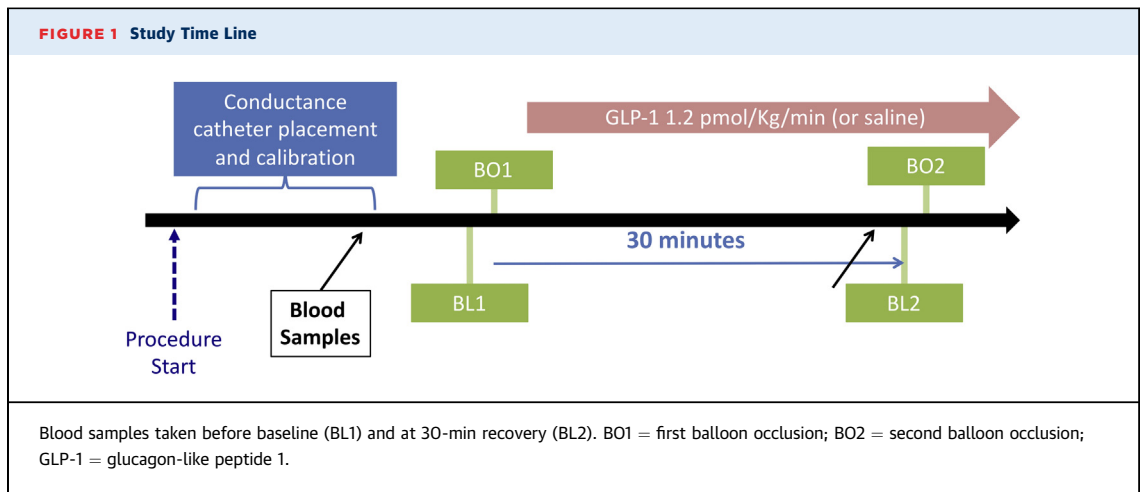
The present study investigated whether RV dysfunction occurs during serial coronary balloon occlusion, assessed by using the gold standard conductance catheter technique (25-28), and whether it is ameliorated by GLP-1. These data will confirm whether GLP-1 cardioprotection is confined to the left ventricle or whether it offers protection from RV ischemia.

## METHODS

**STUDY POPULATION.** Patients with severe, dominant (providing the posterior descending artery) RCA

## ABBREVIATIONS AND ACRONYMS

- BL** = baseline
- BO1** = first balloon occlusion
- BO2** = second balloon occlusion
- dp/dt<sub>max</sub>** = maximal rate of isovolumetric contraction
- dp/dt<sub>min</sub>** = maximal rate of isovolumetric relaxation
- DSHB** = Developmental Studies Hybridoma Bank
- EDP** = end-diastolic pressure
- GLP** = glucagon-like peptide
- GLP-1R** = glucagon-like peptide 1 receptor
- LV** = left ventricular
- PCI** = percutaneous coronary intervention
- PV** = pressure-volume
- RCA** = right coronary artery
- RV** = right ventricular
- Tau** = time constant of diastolic relaxation



disease awaiting single-vessel elective PCI, and with normal RV function assessed by echocardiography, were recruited. Patients were excluded if they had experienced a myocardial infarction in the preceding 3 months, had a pacemaker or significant valvular heart disease, or were not in sinus rhythm. All patients provided written informed consent before study inclusion.

Patients were recruited in 2 blocks (control followed by GLP-1) to test the first hypothesis that serial balloon occlusion caused ischemic dysfunction, before testing whether GLP-1 infusion ameliorated the dysfunction. The study protocol was designed to match that used by Read et al. (11) to assess the effect of GLP-1 on the left ventricle. The study was approved by the local ethics committee (REC 14/EE/0141) and complied with the Declaration of Helsinki. The study was registered on clinicaltrials.gov (NCT02236299); the trial identification number was UKCRN14028.

**PRE-STUDY PROTOCOL.** Variables that could alter coronary or ventricular hemodynamic variables were minimized. Patients were asked to abstain from consuming caffeine, alcohol, and nicotine, as well as nicorandil and oral/sublingual nitrates, in the 24 h before the procedure. Patients were fasted for 6 h, received aspirin 300 mg and clopidogrel 300 mg before the procedure, and were anticoagulated with unfractionated heparin (70 to 100 IU/kg). An activated clotting time was maintained >250 s throughout the procedure.

**CARDIAC CATHETERIZATION.** Figure 1 depicts the study time line. A 6-F sheath was placed in the right radial artery and a 7-F sheath was placed in the right femoral vein under local anesthetic. Glyceryl

trinitrate 100 µg was administered into the radial artery at the beginning of the procedure as standard to prevent radial spasm but not into the coronary arteries. Patients received 500 ml 0.9% saline administered intravenously before the procedure. No other infusions were administered during the procedure. A 6-F multipurpose catheter was positioned in the pulmonary artery and then the right atrium to measure mean pressures and obtain mixed venous blood gas saturations for determination of indirect Fick cardiac output. Blood was sampled to measure blood resistivity. A 7-F eight-electrode conductance catheter (Millar, Inc., Houston, Texas) was connected to an MPVS Ultra (Millar, Inc.) signal-conditioning unit in series with the PowerLab 16/30 (ADInstruments, New South Wales, Australia) 16-channel amplifier. The conductance catheter was submerged in a saline bath and the pressure transducer zeroed before insertion through the venous sheath and positioning it apically along the long axis of the right ventricle under fluoroscopic guidance (Figure 2A). The conductance catheter was calibrated by using the technique first described in the left ventricle by Baan et al. (29) that has subsequently been used for the right ventricle (30,31).

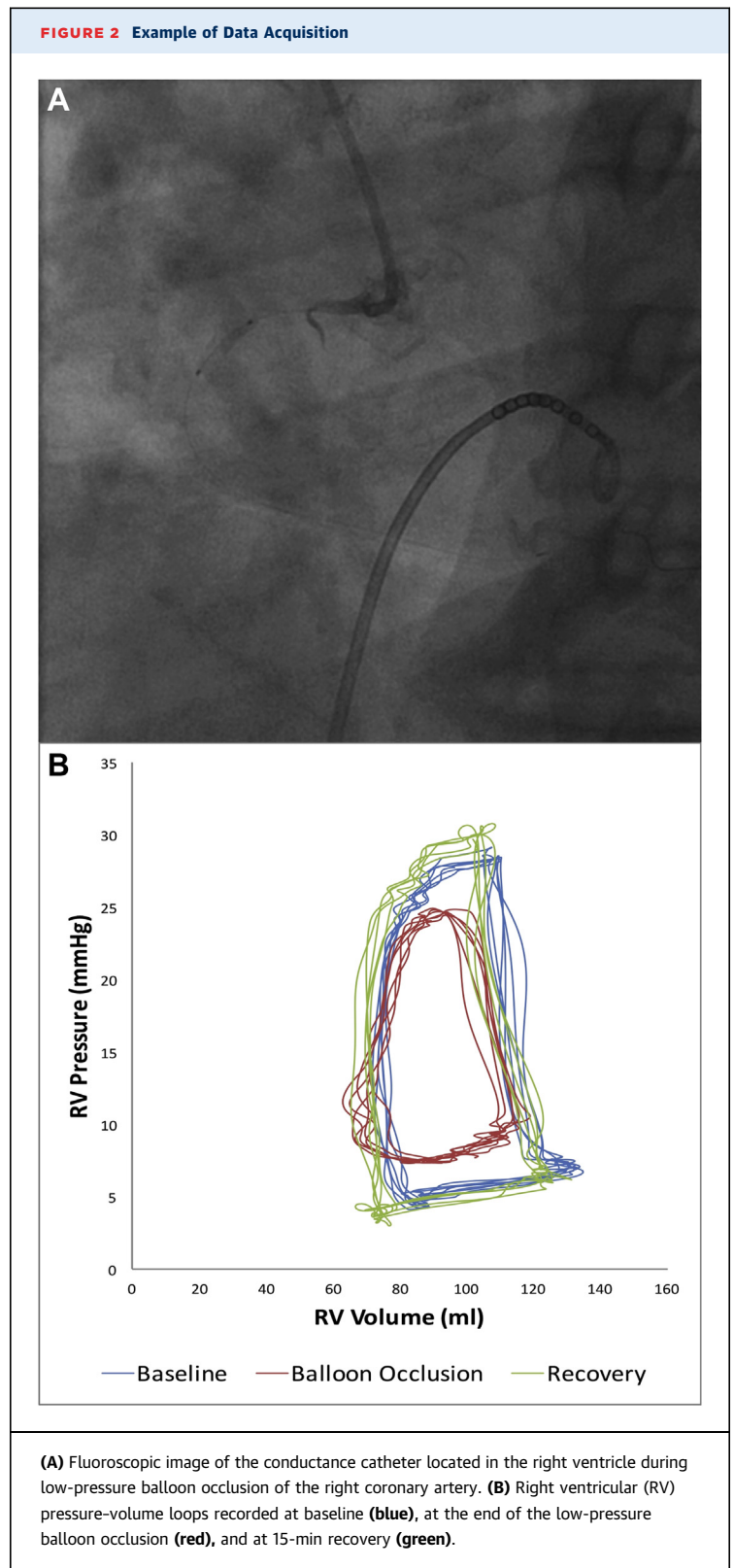
**PRESSURE-VOLUME LOOP DATA ACQUISITION.** The conductance technique was used to measure the pressure-volume (PV) loop relationship during mid-expiration breath hold, providing beat-to-beat assessment of RV function at steady state for at least 5 cardiac cycles. PV loop data were recorded at baseline (BL1), the end of a 1-min low-pressure (<4 atm) balloon occlusion (BO1), and at 1-min recovery. The study infusion was then immediately commenced. PV loop data were acquired after

30-min recovery and at the end of a further 1-min balloon occlusion (BO2). Once data collection was completed, PCI was performed at operator discretion. An example of PV loops generated from the right ventricle during balloon occlusion is shown in **Figure 2B**.

**OFFLINE RV HEMODYNAMIC MEASUREMENTS.** Conductance catheter data were analyzed offline by using LabChart software (LabChart 7.0, ADInstruments). Five steady-state PV loops were recorded at each time point, generating load-dependent parameters of systolic and diastolic function. Systolic parameters of ventricular function were cardiac output, stroke volume, stroke work, ejection fraction, end-systolic pressure, and the maximum rate of isovolumic contraction ( $dp/dt_{max}$ ). Effective arterial elastance to assess afterload was also assessed. Diastolic parameters of ventricular function were end-diastolic pressure (EDP), the maximum rate of isovolumic relaxation ( $dp/dt_{min}$ ), and the time constant of diastolic relaxation (Tau) (32-34). Tau represents the exponential decay of the RV pressure during isovolumic relaxation and was determined by using the Weiss method. Tau is considered load dependent but is predominantly affected by heart rate.

**STUDY INFUSIONS.** Infusion of GLP-1 (7 to 36) amide acetate (or 0.9% saline solution at matched rate) at 1.2 pmol/kg/min was administered after the first balloon occlusion (BO1) until completion of the PV loop measurement (after BO2). This infusion was at the same dose as that administered in previous studies which reduced ischemic dysfunction in the left ventricle (11-13).

**BIOCHEMISTRY.** Baseline peripheral venous blood samples to measure glucose, insulin, GLP-1 (7 to 36) amide, and free fatty acids were obtained at the beginning of the case. Additional peripheral venous blood samples were drawn before the second balloon occlusion. Blood for GLP-1 assays was drawn up into pre-prepared 2-ml syringes containing 20  $\mu$ l of dipeptidyl peptidase-4 inhibitor (Merck Millipore, Nottingham, United Kingdom). These syringes were chilled before collection, and the blood sample was immediately transferred to 2.5-ml ethylenediaminetetraacetic acid tubes, which had also been prepared, containing the protease inhibitor aprotinin (Trasylol, Nordic Group, Trondheim, Norway). These samples were kept in crushed ice until they were spun and stored at  $-20^{\circ}\text{C}$ . Samples for



**TABLE 1** Demographic and Hemodynamic Data

	Control (n = 13)	GLP-1 (n = 11)	p Value
<b>Demographic characteristics</b>			
Age, yrs	72 (62-75)	66 (58-72)	0.25
Male	11 (84.6)	7 (63.6)	0.24
Body mass index, kg/m <sup>2</sup>	28.0 ± 4.0	31.3 ± 5.3	0.10
Smoking history	8 (61.5)	5 (45.4)	0.43
CCS class (II+)	11 (84.6)	7 (63.6)	0.24
NYHA functional class (II or higher)	4 (30.7)	6 (54.5)	0.24
Previous PCI	6 (46.2)	4 (36.3)	0.63
Hypertension	4 (30.7)	3 (27.3)	0.85
Diabetes	1 (7.6)	3 (27.3)	0.20
Previous MI	3 (23.1)	2 (18.1)	0.77
Hemoglobin, g/dl	13.7 ± 1.7	13.7 ± 1.2	0.99
Creatinine, mg/dl	1.0 ± 0.3	1.0 ± 0.2	0.36
<b>Baseline hemodynamic variables</b>			
Systolic blood pressure, mm Hg	136 ± 21	140 ± 27	0.69
Diastolic blood pressure, mm Hg	68 ± 12	69 ± 10	0.98
Systemic MAP, mm Hg	91 ± 13	93 ± 13	0.79
Mean RA pressure, mm Hg	6 (4-8)	4 (3-6)	0.12
Mean PA pressure, mm Hg	18 (17-21)	13 (12-19)	0.07
PA saturations, %	71.6 ± 7.0	71.2 ± 3.6	0.86
Aortic saturations, %	94.6 ± 2.2	96.0 ± 1.4	0.11
Cardiac output, l/min	5.27 ± 1.05	4.88 ± 0.79	0.14
Cardiac index, l/min/kg	2.67 ± 0.51	2.45 ± 0.29	0.07
<b>Baseline hemodynamic variables- RV conductance catheter data</b>			
Stroke work, mm Hg/ml	1,377 ± 575	1,001 ± 382	0.06
Stroke volume, mm Hg/ml	85.9 ± 17.7	81.5 ± 18.6	0.26
End-systolic pressure, mm Hg	28.5 ± 8.7	24.5 ± 7.2	0.26
End diastolic pressure, mm Hg	7.6 ± 3.9	8.6 ± 3.6	0.37
End systolic volume, ml	104 ± 41	81 ± 43	0.19
End diastolic volume, ml	147 ± 40	118 ± 50	0.19
Ejection fraction, %	57.7 ± 9.4	61.7 ± 13.1	0.41
dP/dt <sub>max</sub> , mm Hg/s	360 ± 78	368 ± 116	0.97
dP/dt <sub>min</sub> , mm Hg/s	-259 ± 91	-246 ± 49	0.80
Tau, ms	56 ± 13	68 ± 21	0.06
Ea, mm Hg/ml	0.34 ± 0.09	0.33 ± 0.15	0.98
Values are median (interquartile range), n (%), or mean ± SD.			
CCS = Canadian Cardiovascular Society functional classification of angina; dP/dt <sub>max</sub> = maximum rate of isovolumic contraction; dP/dt <sub>min</sub> = maximum rate of isovolumic relaxation; Ea = effective arterial elastance; GLP-1 = glucagon-like peptide 1; NYHA = New York Heart Association; MI = myocardial infarction; MAP = mean arterial pressure; PA = pulmonary artery; PCI = percutaneous coronary intervention; RA = right atrial; Tau = time constant of diastolic relaxation.			

insulin and free fatty acids were also collected. Blood samples were collected into lithium-heparin tubes, which were also stored on crushed ice before centrifugation and storage at -20°C. All samples were spun within 1 h of collection.

**IMMUNOHISTOCHEMISTRY.** Human tissue samples from anonymous donors were stained for the GLP-1R to correlate our clinical findings with immunohistochemistry. LV and RV samples from nondiabetic patients with ischemic heart disease were

stained. Tissue samples from the Royal Papworth Hospital Tissue Bank were stained for the presence of the GLP-1R. Tissue samples were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer for a minimum of 24 h before dehydration and paraffin embedding. Pancreas was used as a positive control and also stained with hematoxylin-eosin to identify the beta cells. Matched tissue samples from left and right ventricles underwent immunohistochemical analysis using the mAb 3F52 GLP-1R antibody. This receptor was sourced from the University of Iowa Developmental Studies Hybridoma Bank (DSHB); monoclonal antibody (mAb) 3F52 was deposited to the DSHB by Knudsen, L.B. (DSHB Hybridoma Product mAb 3F52). It has previously been validated as specific for the GLP-1R to map GLP-1R expression (35).

**STATISTICAL ANALYSIS.** Data are expressed as mean ± SD unless otherwise stated. Analysis was performed by using SPSS version 25 (IBM SPSS Statistics, IBM Corporation, Armonk, New York). The sample sizes used in the present analysis had the power to detect differences between treatment and placebo groups as estimated by previous research (11). A minimum of 11 patients per group was needed to achieve 80% power. Permission to recruit 15 patients in each group was obtained, ensuring that the study could be completed if datasets were incomplete. Comparison within the groups used a paired Student's *t*-test. For comparisons between groups, nonparametric data were compared by using a Mann-Whitney *U* test, whereas normally distributed data used an unpaired Student's *t*-test. Categorical data were compared with the Fisher exact test. The *p* values <0.05 were considered statistically significant.

## RESULTS

A total of 27 patients were recruited to the study. Three patients were withdrawn from subsequent analysis for technical reasons. Patient demographic data are summarized in **Table 1**. There were no statistically significant differences between the groups, although patients in the GLP-1 group trended toward having increased mean pulmonary artery pressure compared with the control group. Baseline hemodynamic data were broadly similar between groups. However, there was a trend toward increased Tau (*p* = 0.06) and reduced stroke work (*p* = 0.06) in the GLP-1 group.

**TABLE 2 RV Hemodynamic Data at All Study Time Points**

	BL1	BO1	p Value (vs. BL1)	1-min	p Value (vs. BL1)	BL2	p Value (vs. BL1)	BO2	p Value (vs. BO1)
<b>Control group</b>									
Heart rate, beats/min	62 ± 12	58 ± 11	0.17	61 ± 12	0.08	62 ± 10	0.47	59 ± 10	0.28
Stroke work, mm Hg/ml	1,377 ± 575	742 ± 355	<0.01	1,351 ± 688	0.29	954 ± 381	<0.01	745 ± 216	0.94
Cardiac output, l/min	5.3 ± 1.0	3.6 ± 0.8	<0.01	5.0 ± 1.1	0.06	4.6 ± 1.2	0.03	4.1 ± 0.9	0.42
Stroke volume, ml	85.9 ± 17.7	62.6 ± 13.1	<0.001	82.1 ± 17.8	0.28	75.8 ± 17.2	0.06	67.0 ± 16.5	0.48
ESP, mm Hg	28.5 ± 8.7	28.0 ± 9.3	0.58	27.2 ± 12.3	0.57	29.2 ± 11.7	0.54	29.5 ± 9.5	0.03
EDP, mm Hg	7.6 ± 3.9	9.6 ± 4.0	<0.001	7.5 ± 4.2	0.03	9.0 ± 3.3	<0.01	10.8 ± 4.1	0.06
ESV, ml	104.7 ± 40.8	116.6 ± 31.7	0.18	87.5 ± 39.9	0.14	123.9 ± 43.5	0.05	130.8 ± 43.2	0.01
EDV, ml	146.8 ± 40.2	145.8 ± 27.3	0.80	130.3 ± 33.2	0.19	161.0 ± 37.9	0.25	163.6 ± 42.5	0.03
Ejection fraction, %	57.7 ± 11.5	44.3 ± 13.0	<0.01	59.8 ± 18.9	0.08	48.5 ± 10.2	<0.01	43.9 ± 13.6	0.30
dP/dt <sub>max</sub> , mm Hg/s	360 ± 78	297 ± 90	<0.01	411 ± 144	<0.01	326 ± 85	<0.01	276 ± 86	0.01
dP/dt <sub>min</sub> , mm Hg/s	-260 ± 91	-192 ± 76	<0.01	-235 ± 105	0.44	-230 ± 97	<0.01	-192 ± 87	0.99
Tau, ms	55.8 ± 13.4	108.2 ± 43.3	<0.001	69.3 ± 27.5	0.02	72.9 ± 14.0	<0.001	106.0 ± 29.6	0.77
Ea, mm Hg/ml	0.34 ± 0.10	0.50 ± 0.19	0.01	0.32 ± 0.14	0.85	0.41 ± 0.20	0.10	0.47 ± 0.19	0.74
<b>GLP-1 group</b>									
Heart rate, beats/min	62 ± 10	60 ± 8	0.41	63 ± 8	0.71	60 ± 9	0.19	60 ± 7	0.77
Stroke work, mm Hg/ml	1,001 ± 381	878 ± 378	0.14	852 ± 406	0.12	860 ± 374	0.31	787 ± 369	0.18
Cardiac output, l/min	4.9 ± 0.6	4.1 ± 0.8	<0.01	4.4 ± 0.9	0.26	4.3 ± 1.3	0.16	4.1 ± 1.0	0.52
Stroke volume, ml	81.5 ± 18.5	68.3 ± 16.4	0.04	72.2 ± 20.7	0.28	73.9 ± 24.8	0.22	69.1 ± 19.5	0.54
ESP, mm Hg	24.5 ± 7.2	25.7 ± 8.8	0.26	26.5 ± 8.1	0.06	26.3 ± 5.4	0.05	27.1 ± 6.9	0.24
EDP, mm Hg	8.6 ± 3.6	10.2 ± 4.3	0.04	9.8 ± 3.9	0.04	9.7 ± 3.4	0.08	10.4 ± 3.9	0.34
ESV, ml	81.1 ± 43.4	83.9 ± 41.1	0.41	77.0 ± 46.1	0.59	83.2 ± 30.8	0.08	91.6 ± 42.1	0.04
EDV, ml	118.8 ± 50.3	120.7 ± 48.8	0.74	120.9 ± 44.5	0.79	136.3 ± 54.6	0.15	132.0 ± 48.4	0.11
Ejection fraction, %	61.7 ± 13.1	53.4 ± 10.4	0.01	60.8 ± 16.5	0.92	55.9 ± 12.3	0.04	54.9 ± 13.8	0.87
dP/dt <sub>max</sub> , mm Hg/s	368 ± 115	307 ± 86	0.05	346 ± 89	0.50	313 ± 76	0.02	295 ± 73	0.59
dP/dt <sub>min</sub> , mm Hg/s	-246 ± 49	-219 ± 67	0.05	-238 ± 57	0.25	-240 ± 39	0.84	-216 ± 59	0.92
Tau, ms	67.8 ± 21.0	96.5 ± 41.3	0.01	87.4 ± 31.8	<0.01	79.6 ± 22.3	<0.01	99.5 ± 33.9	0.32
Ea, mm Hg/ml	0.33 ± 0.15	0.39 ± 0.23	0.06	0.41 ± 0.24	0.07	0.41 ± 0.21	0.01	0.45 ± 0.29	0.05

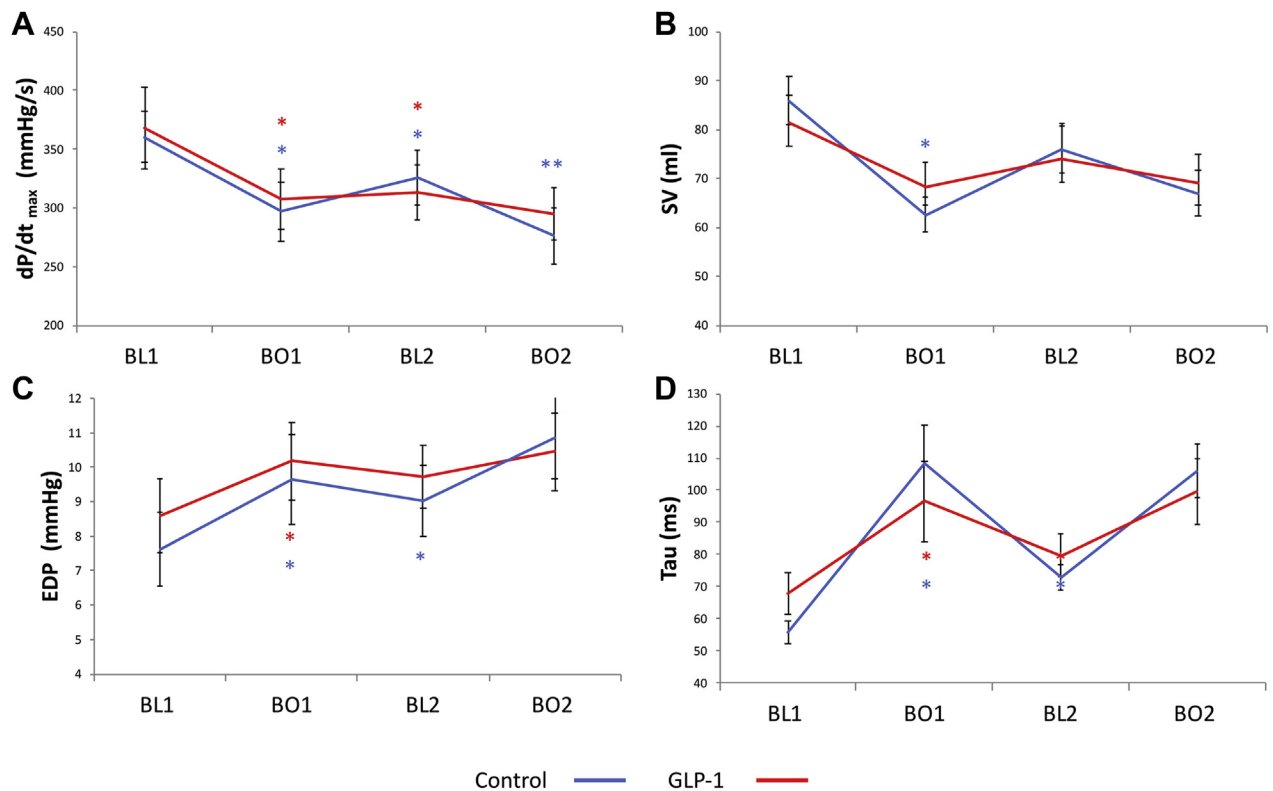
Values are mean ± SD.

1-min = 1-minute recovery; BL1 = baseline; BL2 = 30-min recovery; BO1 = first balloon occlusion; BO2 = second balloon occlusion; ESP = end-systolic pressure; EDP = end-diastolic pressure; ESV = end-systolic volume; EDV = end-diastolic volume; other abbreviations as in Table 1.

**EFFECT OF REPEATED CORONARY BALLOON OCCLUSION ON RV FUNCTION.** Occlusion of the RCA was associated with deterioration of systolic and diastolic function compared with baseline (BL1). At the end of the first balloon occlusion (BO1), stroke volume, ejection fraction, and dP/dt<sub>max</sub> were significantly reduced, with Tau and EDP increased (Table 2). Systolic function improved modestly after 1 min of reperfusion, and only dP/dt<sub>max</sub> improved to above baseline function. Similarly, there were modest improvements in diastolic function at the 1-min recovery, but Tau was still significantly impaired compared with baseline. At 30-min recovery (BL2), there was numerical improvement compared with BO1 in most measures of systolic and diastolic function, with stroke volume (p = 0.08), dP/dt<sub>max</sub> (p = 0.07), and dP/dt<sub>min</sub> (p = 0.09) trending toward improvement, and a statistically significant improvement in Tau (p < 0.01). Nonetheless, most measures remained

impaired compared with BL1 (cardiac output, stroke work, ejection fraction, dP/dt<sub>max</sub>, dP/dt<sub>min</sub>, EDP, and Tau), suggesting that there was stunning of the right ventricle at the 30-min recovery. Further balloon occlusion (BO2) was associated with impairment of the right ventricle, but only dP/dt<sub>max</sub> (p = 0.01) showed significant impairment of function compared with BO1, consistent with cumulative ischemic RV dysfunction.

**EFFECT OF GLP-1 ON RV FUNCTION DURING BALLOON OCCLUSION.** The change in parameters of RV function in the GLP-1 group was similar to those of the saline control group, with systolic and diastolic dysfunction after BO1 (before starting the GLP-1 infusion), stunning, and cumulative ischemic RV dysfunction observed (Table 2). There was no significant difference in any marker of systolic or diastolic function between the saline and GLP-1 groups at

**FIGURE 3** Serial RV Hemodynamic Data

BO1 caused significant reduction in the (A) maximum rate of isovolumic contraction ( $dP/dt_{max}$ ) and (B) stroke volume (SV) and (C) increases in end-diastolic pressure (EDP) and (D) the time constant of diastolic relaxation (Tau). \* $p < 0.05$  versus BL1. Cumulative ischemic dysfunction measured according to  $dP/dt_{max}$  after a second balloon occlusion was observed. \*\* $p < 0.05$  versus BO1. There was no significant difference in any right ventricular index between GLP-1 and control saline. Mean  $\pm$  SEM. Compared by using Student's *t*-test. GLP-1,  $n = 11$ ; control,  $n = 13$ . Abbreviations as in Figure 1.

either 30-min recovery or the second balloon occlusion (Figure 3).

**BIOCHEMISTRY.** Figure 4 shows that GLP-1 levels rose in the GLP-1-infused arm while remaining unchanged in the control arm. GLP-1 was metabolically active, causing a significant rise in insulin levels and a fall in plasma glucose levels. There was a small, but significant, drop in insulin levels in the control group. This reduction may represent the fasted nature of the cohort. However, there were no hypoglycemic episodes recorded during the study. Plasma free fatty acids rose in both groups as a result of the administration of unfractionated heparin required for the procedure (36), but there were no significant differences in free fatty acid levels between the groups.

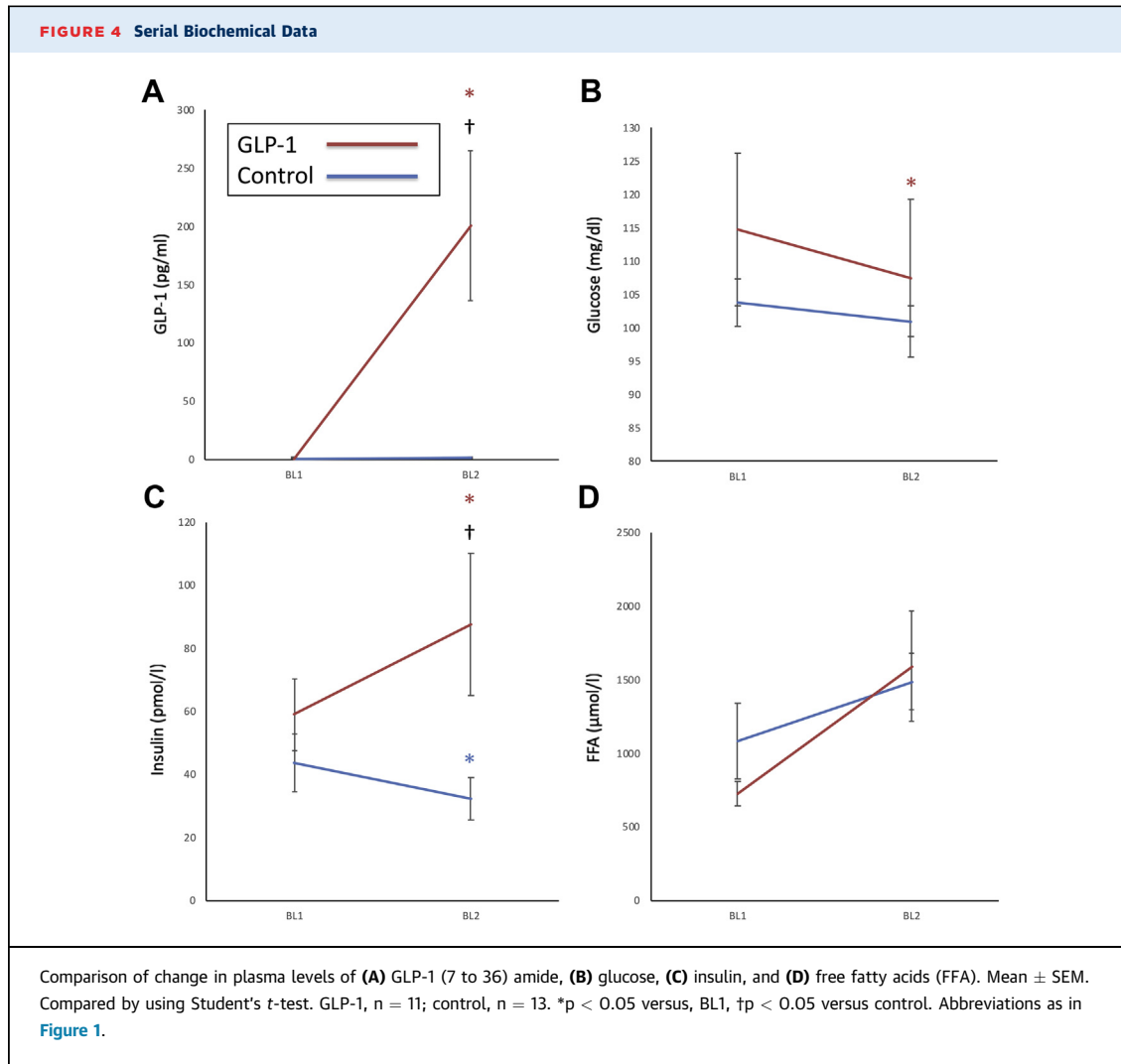
**IMMUNOHISTOCHEMISTRY.** Antibody staining of human RV and LV tissue confirmed patchy mAb

3F52 binding to cardiomyocytes, indicating the presence of the GLP-1R in both ventricles (Figure 5).

## DISCUSSION

To the best of our knowledge this study is the first, to assess the effect of GLP-1 on RV function using the conductance technique in humans during supply ischemia precipitated by repeat coronary balloon occlusion. RCA occlusion was associated with marked deterioration in systolic and diastolic measures of RV function. There was rapid RV recovery of some indices at 1 min, although residual stunning was observed at 30 min. Further occlusion was associated with cumulative RV dysfunction in some indices. GLP-1 did not abrogate myocardial stunning or ischemic RV dysfunction.

Ischemic LV dysfunction and stunning after transient coronary balloon occlusion have been reported



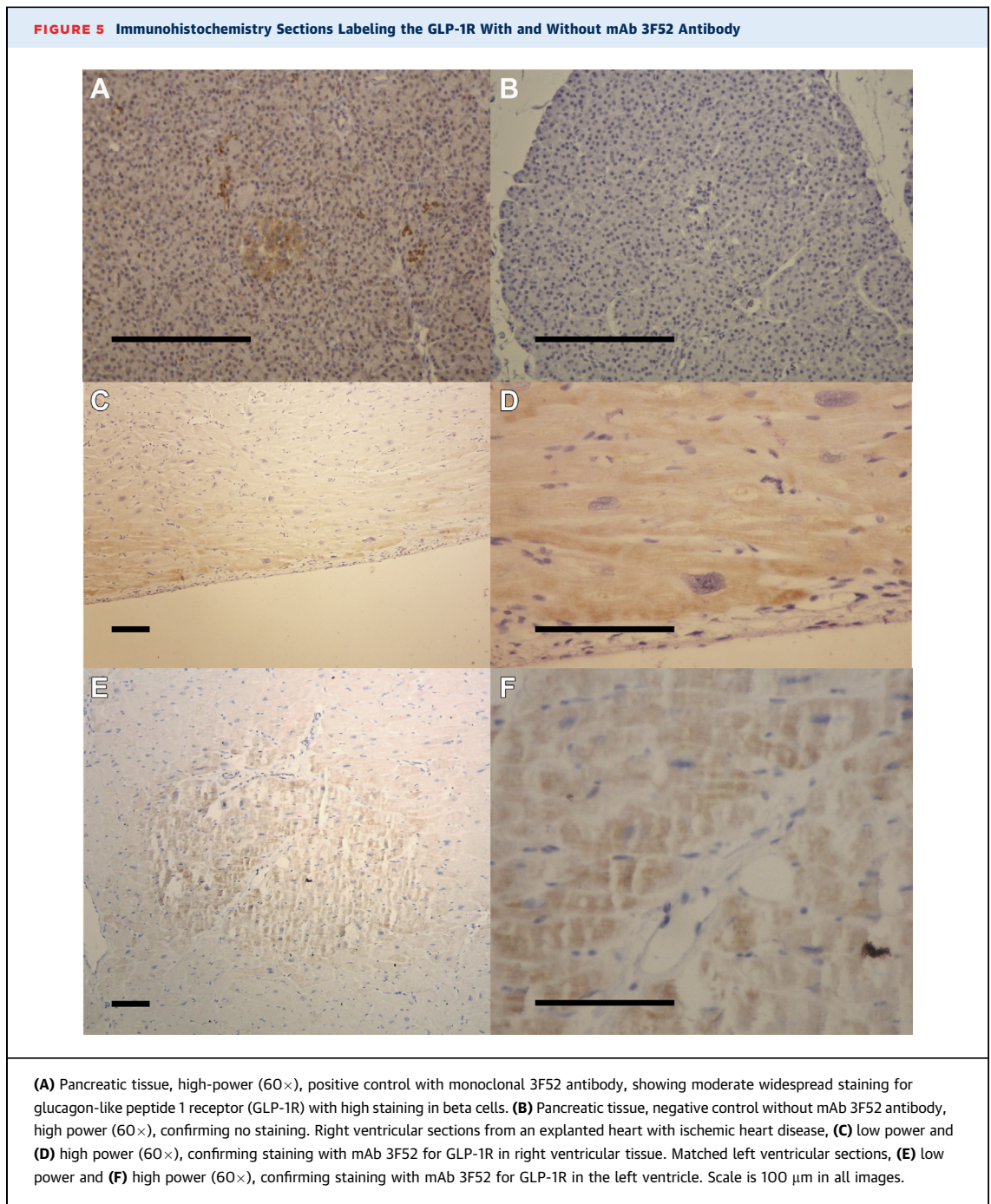
previously by our group (11-13). Transient supra-baseline improvement in systolic performance occurs during early reperfusion due to reactive hyperemia, causing increased coronary flow that augments LV function through a phenomenon known as the Gregg effect (37). Increased volume of the microvasculature after reperfusion causes stretch-activated calcium channels to open. The resultant influx of calcium increases myocyte contractility and briefly masks the effect of ischemic LV dysfunction, despite the presence of stunning (38). Stunning is revealed when the reactive hyperemia subsides.

In the present study, the magnitude of the effect of coronary balloon occlusion and reperfusion on RV function was blunted compared with studies investigating the left ventricle. This difference may be explained by: 1) the comparatively low myocardial

mass of the right ventricle; 2) reduced ischemic burden during RCA occlusion; 3) the conduit nature of the right ventricle as a volume pump; and 4) because up to 50% of RV function is derived from the left ventricle, through a shared septum and ventricular interdependence. Nevertheless, in contrast to previous studies (6), we assessed the prolonged recovery of the right ventricle from transient supply ischemia. We confirmed that, like the left ventricle, when reactive hyperemia subsides, RV stunning is discernible and cumulative RV dysfunction can be observed.

GLP-1 abrogates LV stunning and cumulative ischemic dysfunction during both supply (coronary artery occlusion) and demand (dobutamine stress) ischemia, in a consistent manner (11,13,39). The absence of a cardioprotective effect in the right





ventricle is surprising, particularly as we have confirmed that GLP-1 levels were significantly augmented in our study and that the GLP-1R is expressed on RV myocytes. This finding is consistent with other recently published data showing the presence of GLP-1R messenger ribonucleic acid in all 4 chambers of the heart (40). The absence of protective effect may again be explained by the reduced

mass of the right ventricle. Although GLP-1 still binds to cardiomyocytes in the right ventricle, the effect size could be too small to be detected clinically by the conductance catheter. Furthermore, the reduced myocardial mass of the right ventricle may prevent the detection of cardioprotection by GLP-1 in this thinner walled ventricle. The GLP-1R appears to be expressed in the same density as in the left ventricle,

although we have not been able to accurately quantify receptor density for comparison in this study. It is possible that although GLP-1 binds the receptor in the right ventricle, this action does not affect the RV cardiomyocytes in the same fashion as in the left ventricle. The presence of persistent RV impairment after PCI to the RCA is pertinent to clinical practice.

Our findings may be especially relevant in patients with limited RV functional reserve, in whom hemodynamic instability after PCI is a particular risk. Minimizing the duration of coronary balloon occlusion during PCI in this subset of patients could reduce the risk of hemodynamic compromise. From a translational perspective, GLP-1 and GLP-1R agonists remain potential therapeutic agents for those with acute hemodynamic disturbance related to myocardial ischemia. Pilot studies have shown that GLP-1R agonists reduce the need for inotropic support for critically ill patients (41,42). However, our data suggest that, although GLP-1 may be a possible therapy for ischemic LV dysfunction, GLP-1 is not likely to be a useful therapy for reducing ischemic RV dysfunction.

**STUDY LIMITATIONS.** The 30-min recovery period was chosen for ethical and practical reasons. Longer follow-up to show that parameters eventually returned to baseline values would be desirable to confirm the reversible nature of RV stunning. Similarly, we did not directly confirm coronary flow normalization required to fulfill the definition of stunning. However, we and others have confirmed recovery of basal flow velocity within this time frame in the left coronary artery (8). The myocardial bed subtended by the RCA is smaller, and therefore, a priori, the ischemic insult after RCA occlusion is less and subsequent reactive hyperemia in response shorter than that seen after left coronary artery occlusion. Despite recruiting patients with proximal stenoses in dominant RCAs, we did not confirm the degree of ischemic insult by using another modality (e.g., serum lactate). It is possible that the 2 groups had different ischemic burdens that masked any difference being observed in the GLP-1 group.

The right ventricle is a challenging chamber to assess in all imaging modalities. For RV conductance studies, its thin wall increases parallel conductance, whereas its eccentric shape means that volume assessment is less amenable to simple geometric modeling than the conical left ventricle. Nonetheless, a number of studies have shown that RV conductance studies provide accurate assessment of the right ventricle (6,26,27).

Patients in the present study were not randomized to treatment. However, all eligible patients were

consecutively recruited into the study from the elective PCI waiting list compiled independently from the clinicians involved in the study. The endpoint data were objective empiric hemodynamic data and not influenced by knowledge of the allocation and techniques employed were familiar to the operators, minimizing the risk of a “learning curve” on the results. GLP-1 protects against ischemic left ventricular dysfunction. There was a nonsignificant difference in the baseline characteristics of the 2 groups that was unexpected and may have disadvantaged the GLP-1 group and prevented small improvements in RV dysfunction being observed after GLP-1 compared with control subjects. However, patients also acted as their own control with serial BO, and we believe a neutral effect of GLP-1 on the right ventricle is likely.

## CONCLUSIONS

Stunning and cumulative ischemic RV dysfunction was observed after RCA balloon occlusion in human subjects. This scenario may contribute to hemodynamic instability in patients with limited RV reserve. GLP-1 infusion did not attenuate this ischemic RV dysfunction.

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## PERSPECTIVES

**COMPETENCY IN MEDICAL KNOWLEDGE:** Animal studies have shown that GLP-1 protects against lethal ischemia-reperfusion injury. Human studies have shown that GLP-1 protects against ischemic left ventricular dysfunction. This translational study found that stunning and cumulative dysfunction occur in the right ventricle but that GLP-1 does not abrogate this action. These findings may be of clinical relevance to a subset of patients with limited RV reserve during PCI.

**TRANSLATIONAL OUTLOOK:** Additional research is needed to address the mechanisms behind GLP-1 cardioprotection in the left ventricle and whether GLP-1 also protects against lethal ischemia-reperfusion injury in humans.

## REFERENCES

- Haddad F, Hunt SA, Rosenthal DN, Murphy DJ. Right ventricular function in cardiovascular disease, part I: anatomy, physiology, aging, and functional assessment of the right ventricle. *Circulation* 2008;117:1436-48.
- O'Rourke RA, Dell'Italia LJ. Diagnosis and management of right ventricular myocardial infarction. *Curr Probl Cardiol* 2004;29:6-47.
- de Groote P, Millaire A, Foucher-Hossein C, et al. Right ventricular ejection fraction is an independent predictor of survival in patients with moderate heart failure. *J Am Coll Cardiol* 1998;32:948-54.
- Farrer-Brown G. Vascular pattern of myocardium of right ventricle of human heart. *Br Heart J* 1968;30:679-86.
- Haupt HM, Hutchins GM, Moore GW. Right ventricular infarction: role of the moderator band artery in determining infarct size. *Circulation* 1983;67:1268-72.
- Bishop A, White P, Groves P, et al. Right ventricular dysfunction during coronary artery occlusion: pressure-volume analysis using conductance catheters during coronary angioplasty. *Heart* 1997;78:480-7.
- Axell RG, Giblett JP, White PA, et al. Stunning and right ventricular dysfunction is induced by coronary balloon occlusion and rapid pacing in humans: insights from right ventricular conductance catheter studies. *J Am Heart Assoc* 2017;6.
- Hoole SP, Heck PM, White PA, et al. Stunning and cumulative left ventricular dysfunction occurs late after coronary balloon occlusion in humans insights from simultaneous coronary and left ventricular hemodynamic assessment. *J Am Coll Cardiol Intv* 2010;3:412-8.
- Marso SP, Daniels GH, Brown-Frandsen K, et al. Liraglutide and cardiovascular outcomes in type 2 diabetes. *N Engl J Med* 2016;375:311-22.
- Marso SP, Bain SC, Consoi A, et al. Semaglutide and cardiovascular outcomes in patients with type 2 diabetes. *N Engl J Med* 2016;375:1834-44.
- Read PA, Hoole SP, White PA, et al. 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;4:266-72.
- McCormick LM, Hoole SP, White PA, et al. Pre-treatment with glucagon-like peptide-1 protects against ischemic left ventricular dysfunction and stunning without a detected difference in myocardial substrate utilization. *J Am Coll Cardiol Intv* 2015;8:292-301.
- Giblett JP, Axell RG, White PA, et al. Glucagon-like peptide-1 derived cardioprotection does not utilize a KATP-channel dependent pathway: mechanistic insights from human supply and demand ischemia studies. *Cardiovasc Diabetol* 2016;15:99.
- Bose AK, Mocanu MM, Carr RD, Brand CL, Yellon DM. Glucagon-like peptide 1 can directly protect the heart against ischemia/reperfusion injury. *Diabetes* 2005;54:146-51.
- Bose AK, Mocanu MM, Carr RD, Yellon DM. Glucagon like peptide-1 is protective against myocardial ischemia/reperfusion injury when given either as a preconditioning mimetic or at reperfusion in an isolated rat heart model. *Cardiovasc Drugs Ther* 2005;19:9-11.
- Bose AK, Mocanu MM, Carr RD, Yellon DM. Myocardial ischaemia-reperfusion injury is attenuated by intact glucagon like peptide-1 (GLP-1) in the in vitro rat heart and may involve the p70s6K pathway. *Cardiovasc Drugs Ther* 2007;21:253-6.
- Nikolaidis LA, Elahi D, Shen YT, Shannon RP. Active metabolite of GLP-1 mediates myocardial glucose uptake and improves left ventricular performance in conscious dogs with dilated cardiomyopathy. *Am J Physiol Heart Circ Physiol* 2005;289:H2401-8.
- Kavianipour M, Ehlers MR, Malmberg K, et al. Glucagon-like peptide-1 (7-36) amide prevents the accumulation of pyruvate and lactate in the ischemic and non-ischemic porcine myocardium. *Peptides* 2003;24:569-78.
- Hausenloy DJ, Whittington HJ, Wynne AM, et al. Dipeptidyl peptidase-4 inhibitors and GLP-1 reduce myocardial infarct size in a glucose-dependent manner. *Cardiovasc Diabetol* 2013;12:154.
- Bao W, Aravindhan K, Alsaied H, et al. Albiglutide, a long lasting glucagon-like peptide-1 analog, protects the rat heart against ischemia/reperfusion injury: evidence for improving cardiac metabolic efficiency. *PLoS One* 2011;6:e23570.
- Aravindhan K, Bao W, Harpel MR, Willette RN, Lepore JJ, Jucker BM. Cardioprotection resulting from glucagon-like peptide-1 administration involves shifting metabolic substrate utilization to increase energy efficiency in the rat heart. *PLoS One* 2015;10:e0130894.
- Halbirk M, Norrelund H, Moller N, et al. Cardiovascular and metabolic effects of 48-h glucagon-like peptide-1 infusion in compensated chronic patients with heart failure. *Am J Physiol Heart Circ Physiol* 2010;298:H1096-102.
- Gejl M, Lerche S, Mengel A, et al. Influence of GLP-1 on myocardial glucose metabolism in healthy men during normo- or hypoglycemia. *PLoS One* 2014;9:e83758.
- Clarke SJ, Giblett JP, Yang LL, et al. GLP-1 is a coronary artery vasodilator in humans. *J Am Heart Assoc* 2018;7:e010321.
- Axell RG, Hoole SP, Hampton-Till J, White PA. RV diastolic dysfunction: time to re-evaluate its importance in heart failure. *Heart Fail Rev* 2015;20:363-73.
- Bishop A, White P, Chaturvedi R, Brookes C, Redington A, Oldershaw P. Resting right ventricular function in patients with coronary artery disease: pressure volume analysis using conductance catheters. *Int J Cardiol* 1997;58:223-8.
- White PA, Bishop AJ, Conroy B, Oldershaw PJ, Redington AN. The determination of volume of right ventricular casts using a conductance catheter. *Eur Heart J* 1995;16:1425-9.
- Aguero J, Ishikawa K, Hadri L, et al. Intratracheal gene delivery of SERCA2a ameliorates chronic post-capillary pulmonary hypertension: a large animal model. *J Am Coll Cardiol* 2016;67:2032-46.
- Baan J, van der Velde ET, de Bruin HG, et al. Continuous measurement of left ventricular volume in animals and humans by conductance catheter. *Circulation* 1984;70:812-23.
- McKay RG, Spears JR, Aroesty JM, et al. Instantaneous measurement of left and right ventricular stroke volume and pressure-volume relationships with an impedance catheter. *Circulation* 1984;69:703-10.
- McCabe C, White PA, Hoole SP, et al. Right ventricular dysfunction in chronic thromboembolic obstruction of the pulmonary artery: a pressure-volume study using the conductance catheter. *J Applied Physiol* 2014;116:355-63.
- Weiss JL, Frederiksen JW, Weisfeldt ML. Hemodynamic determinants of the time-course of fall in canine left ventricular pressure. *J Clin Invest* 1976;58:751-60.
- Raff GL, Glantz SA. Volume loading slows left ventricular isovolumic relaxation rate. Evidence of load-dependent relaxation in the intact dog heart. *Circulation Res* 1981;48:813-24.
- Matsubara H, Takaki M, Yasuhara S, Araki J, Suga H. Logistic time constant of isovolumic relaxation pressure-time curve in the canine left ventricle. Better alternative to exponential time constant. *Circulation* 1995;92:2318-26.
- Pyke C, Heller RS, Kirk RK, et al. GLP-1 receptor localization in monkey and human tissue: novel distribution revealed with extensively validated monoclonal antibody. *Endocrinology* 2014;155:1280-90.
- Nelson PG. Effect of heparin on serum free-fatty-acids, plasma catecholamines, and the incidence of arrhythmias following acute myocardial infarction. *Br Med J* 1970;3:735-7.
- Gregg DE. Effect of coronary perfusion pressure or coronary flow on oxygen usage of the myocardium. *Circ Res* 1963;13:497-500.
- Stahl LD, Aversano TR, Becker LC. Selective enhancement of function of stunned myocardium by increased flow. *Circulation* 1986;74:843-51.
- 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.

40. Baggio LL, Yusta B, Mulvihill EE, et al. GLP-1 receptor expression within the human heart. *Endocrinology* 2018;159:1570-84.

41. Galiatsatos P, Gibson BR, Rabiee A, et al. The glucoregulatory benefits of glucagon-like

peptide-1 (7-36) amide infusion during intensive insulin therapy in critically ill surgical patients: a pilot study. *Crit Care Med* 2014;42:638-45.

42. Sokos GG, Bolukoglu H, German J, et al. Effect of glucagon-like peptide-1 (GLP-1) on glycemic control and left ventricular function in patients

undergoing coronary artery bypass grafting. *Am J Cardiol* 2007;100:824-9.

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