Glucagon-like peptide-1 preserves coronary microvascular endothelial function after cardiac arrest and resuscitation: potential antioxidant effects

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previously described (28, 55, 56, 58). Anesthesia was induced with 5% isoflurane inhalation anesthesia in oxygen administered by nose cone. An endotracheal tube was placed per os, and a surgical plane of anesthesia was maintained by using 1.5–3% isoflurane in room air delivered via a rate- and volume-regulated ventilator/anesthesia machine (Narcomed 2A; North American Drager, Telford, PA). Rate and/or tidal volume was adjusted to maintain end-tidal concentration of carbon dioxide (ETP\textsubscript{CO}\textsubscript{2}) at 40 ± 3 mmHg. Heart rate, aortic pressure, electrocardiogram, end-tidal partial pressure of carbon dioxide (ETP\textsubscript{CO}\textsubscript{2}), and tidal volume were continuously projected on a physiologic recorder (Ponemah Physiology Platform, Model P3 Plus; Gould Instrument Systems, Valley View, OH) for instantaneous visual monitoring and postexperimental data analyses. Vascular sheaths (7F Cordis) were placed in the carotid artery and external jugular vein via cutdown procedure to guide subsequent intravenous catheter placement. For coronary sinus blood draws, the external jugular catheter was advanced into the sinus. Otherwise, catheters were secured in place for the remainder of the experimental protocol.

**Hemodynamic data acquisition.** LV ejection fraction (EF) was measured pre- and postarrest by using contrast left ventriculography. The time constant of isovolumic LV relaxation [Tau (τ)] was obtained by using a high-fidelity, micromanometer-tipped pressure transducer (MPC-500; Millar Instruments, Houston, TX) placed in the LV. Cardiac output, in liters per min, was determined using a thermal dilution technique. Hemodynamic data were acquired (DAQ; Millar Instruments) and analyzed (Data Measuring Unit, MPNVs; Millar Instruments) by commercially available software programs. A 5F Amplatz modified right coronary catheter was used to guide a 0.014-in intracoronary Doppler flow wire (FloWire; Volcano Therapeutics, Rancho Cordova, CA) into the left anterior descending coronary artery. Baseline Doppler flow velocity measurements were made, followed by an intracoronary injection of the endothelium-dependent vasodilator Substance P (Substance P acetate salt hydrate, 40 pmol over 2 min; Sigma Chemical, St. Louis, MO) (43, 71), after which the flow measurements were repeated. Baseline and post-Substance P flow measurements were repeated twice, and the mean of the three measurements was reported for each time point (baseline and 1 and 4 h after ROSC). Data were recorded for later calculation of CFR by using the Flo-Map system (CardioMetrics Flo-Map; EndoSonics, Rancho Cordova, CA).

**Experimental protocol and treatment.** In the meantime, 96 swine were blindly randomized to treatment or placebo (NS) group consisting of nine animals each. After the acquisition of pre-arrest baseline data and blood samples, ventilatory fibrillation (VF) was electrically induced by passing alternating current (60 cycles per s) through the electrodes of a catheter (locally made) in the right ventricle attached to a standard pacing wire. Confirmation of VF was observed by the characteristic ECG waveform and the loss of aortic pressure. Ventilation and anesthesia were discontinued at this time. The animals underwent an 8-min period of untreated VF before initiation of cardiopulmonary resuscitation efforts, consisting of continuous chest compressions at 100 per min and oxygen at 10 ventilator-administered breaths per min. One milligram of epinephrine was given intravenously at the beginning of chest compressions. Cardiopulmonary resuscitation was performed for 90 s after which a single biphasic defibrillation shock of 150 J was administered. Additional epinephrine administrations every 3 min and shocks every 2 min were given if VF was not terminated previously. ROSC was defined as a peak systolic aortic pressure (AoP) of >50 mmHg and a pulse pressure (aortic systolic minus diastolic pressure) of >20 mmHg for 1 min. One minute after ROSC, an infusion pump (Alaris; Cardinal Health, Dublin, OH) was used to begin a 4-h continuous infusion with either human rGLP-1 (American Peptide, 10 pmol kg\textsuperscript{-1} min\textsuperscript{-1}) or equal volume of 0.9% normal saline (NS) as placebo. The animals were monitored continuously, and data measurements were periodically taken as described above. Anesthesia was resumed when spontaneous movements were observed. Four hours after ROSC, final data were collected and animals were de-instrumented (with the exception of 1 venous port), allowed to recover from anesthesia and placed in observation cages. Twenty-four hours post-ROSC a neurological function exam was conducted and the animals were humanely euthanized by intravenous injection of a commercial solution (Fatal-Plus; Vortech, Dearborn, MI).

**Measurement of whole blood glucose and plasma insulin.** Whole blood was drawn from indwelling catheters placed in the carotid artery and also the carotid sinus, pre-arrest, and 30 min and 1, 2, and 4 h after ROSC. Whole blood glucose (in mg/dl) was measured (in duplicate) in blood drawn from the carotid artery immediately after acquisition, using a commercially available handheld monitor (AccuChek Aviva; Roche Diagnostics, Mannheim, Germany). Remaining whole blood from both sites was centrifuged (1,500 g) for 5 min, and the serum was flash frozen in liquid nitrogen and stored at −80°C. Serum insulin concentration was subsequently measured in samples from the carotid artery, by using a commercially available porcine insulin ELISA (Alpco Diagnostics, Salem, NH).

**Measurement of plasma 8-iso-PGF\textsubscript{2a} and SOD activity.** Both systemic (drawn from carotid artery) and coronary sinus samples were used for these assays. Serum concentration of 8-iso-PGF\textsubscript{2a} was determined using a commercially available enzyme-linked immunoblotting assay kit (Cayman Chemical, Ann Arbor, MI). In serum, 8-iso-PGF\textsubscript{2a} may be identified in lipids within the sample and will not be detected without hydrolysis. To obtain the total 8-isoprostane content (vs. free 8-iso-PGF\textsubscript{2a} fractions), serum samples were hydrolyzed with 15% potassium hydroxide followed by neutralization with a potassium phosphate buffer (pH 7.20). Subsequently, enzyme-linked immunoblotting assay for 8-iso-PGF\textsubscript{2a} concentration was performed according to manufacturer instructions on hydrolyzed serum samples. SOD activity in serum was determined by measuring the dismutation of superoxide radicals generated by xanthine oxidase and hypoxanthine, using a commercially available kit (Cayman Chemical), according to manufacturer instructions. Both assays were performed in 96-well plates and absorbance determined by a Varioskan Flash Multimode plate reader (Thermo Scientific), read at 410 and 450 nm for 8-iso-PGF\textsubscript{2a} and SOD activity, respectively.

**Cyclooxygenase-2 protein expression.** Twenty-four hours after ROSC, swine were euthanized by injecting a commercially available solution through the indwelling central venous catheter. Animals were quickly placed on a surgical table, the chest was opened, and the heart and a portion of the thoracic aorta were removed. Sections of LV and aorta were immediately flash-frozen and stored at −80°C. Subsequently, tissues were separately homogenized in 8 vol of ice-cold lysis buffer containing 50 mM HEPES, 150 mM NaCl, 20 mM sodium pyrophosphate, 20 mM β-glycerophosphate, 10 mM Na\textsubscript{2}HPO\textsubscript{4}, 2 mM EDTA, 1% Triton X-100, 10% glycerol, 1 mM MgCl\textsubscript{2}, 1 mM CaCl\textsubscript{2}, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 0.5 μg/ml pepstatin, and 2 mM phenylmethylsulfonyl fluoride. Homogenates were incubated on ice for 20 min and then centrifuged at 13,000 g for 20 min at 4°C. Total protein concentration was determined using the BCA method (Sigma Chemical). For determination of cyclooxygenase (COX)-2 expression, samples containing equal amounts of total protein were separated by SDS-PAGE on 10% polyacrylamide gels (Bio-Rad Laboratories, Hercules, CA) and transferred to nitrocellulose membranes. Membranes were incubated with antibodies against swine COX-2 (Everest Biotech) overnight. Subsequently, membranes were incubated with secondary rabbit anti-goat antibody conjugated with horseradish peroxidase (Everest Biotech). The proteins were visualized with an imaging system (Bio-Rad Chemi-Doc) after exposure to enhanced chemiluminescence detection reagents (Amer sham Pharmacia, Piscataway, NJ). The band intensities on the images were quantified by using Quantity One software (Bio-Rad).

**Data analysis.** Data analyses were performed using commercially available software (SPSS, version 19.0; SPSS) and reviewed by a biostatistician. Prearrest hemodynamic measurements (5 time points) were compared between treatment groups using repeated-measures ANOVA. CFR measurements were compared between treatment groups using a Wilcoxon rank-sum analysis to determine a change in
GLP-1 treatment did not improve survival. Eighteen swine were enrolled in the study. Ten animals completed the study protocol (n = 5 in each treatment group) and were included in final data analyses. Eight animals were excluded during the study protocol due to either 1) repeated, intractable VF or pulseless electrical activity; three animals before randomization, three NS animals, and one GLP-1 animal during the 4-h treatment period; or 2) expiration between 4 and 24 h post-ROSC due to unknown causes (1 GLP-1 animal was found expired during the monitoring period, 12 h after ROSC). All 10 animals that completed the protocol and survived for 24 h after ROSC were neurologically normal. Animals did not differ significantly in weight or sex between treatment groups.

No difference in resuscitation variables between treatment groups. Of the 10 animals that completed the study protocol, there were no differences in length of VF, amount of epinephrine used, or the number of shocks administered before achievement of ROSC (Table 1).

**RESULTS**

GLP-1 treatment did not improve post-ROSC LV function. Hemodynamic and LV function measurements are shown in Table 2. After cardiac arrest and resuscitation, there were significant decreases in LV function. Ejection fraction in both groups was decreased from baseline at all post-ROSC time points (P < 0.05). Although GLP-1-treated animals had statistically better rate of decrease of LV pressure (−dP/dt) at 1 and 4 h post-ROSC, and better relaxation τ at 1 h, the treatment groups were comparable at other time points. Post-ROSC aortic systolic and diastolic pressures declined from baseline in the NS group, but this was not observed in the GLP-1 group (P < 0.05).

GLP-1 treatment did not affect whole blood glucose or plasma insulin concentrations. GLP-1 is a potentiator of glucose-induced insulin secretion, and insulin has vasculo- and cardio-protective properties. In the present study, no change in glucose or insulin concentration from baseline was observed in either group (Table 3). In addition, no significant difference was found in either plasma insulin or blood glucose concentrations between groups at any timepoint.

GLP-1 treatment decreased oxidant injury as measured by plasma 8-iso-PGF_{2α}. In plasma drawn from the coronary sinus 4 h after ROSC, 8-iso-PGF_{2α} was significantly elevated in the control (NS) group from the pre-arrest baseline at both 1 and 4 h after ROSC (Fig. 1). This post-ROSC decline reflects coronary microvascular endothelial dysfunction in the control group, which was not observed in the GLP-1 group (P < 0.05).

**Table 1. Resuscitation variables were similar between treatment groups**

<table>
<thead>
<tr>
<th>Resuscitation Variables</th>
<th>NS</th>
<th>GLP-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shocks, mean number</td>
<td>1.2 ± 0.2</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>Epinephrine, mg</td>
<td>6.0 ± 1.2</td>
<td>8.0 ± 1.6</td>
</tr>
<tr>
<td>Time in ventricular fibrillation, s</td>
<td>603 ± 44</td>
<td>577 ± 55</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 for normal saline (NS) and glucagon-like peptide-1 (GLP-1).

**Table 2. Hemodynamic and prearrest (baseline) variables in NS vs. GLP-1**

<table>
<thead>
<tr>
<th>NS</th>
<th>Baseline</th>
<th>30 Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>119 ± 8.7</td>
<td>132 ± 2.1</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>2.8 ± 0.9</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td>AoS, mmHg</td>
<td>80 ± 2.1</td>
<td>72 ± 3.6</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>53 ± 0.6</td>
<td>44.4 ± 3.2*</td>
</tr>
<tr>
<td>dp/dt</td>
<td>9.2 ± 0.14</td>
<td>10.9 ± 1.0</td>
</tr>
<tr>
<td>−dp/dt</td>
<td>1,335 ± 68</td>
<td>1,234 ± 140</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>0.57 ± 0.03</td>
<td>0.31 ± 0.01*</td>
</tr>
<tr>
<td>τ, ms</td>
<td>45 ± 4</td>
<td>59.3 ± 4.2*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GLP-1</th>
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<tr>
<td>HR, beats/min</td>
<td>109 ± 1.2</td>
<td>140 ± 9.3</td>
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<tr>
<td>CO, l/min</td>
<td>2.7 ± 0.2</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>AoS, mmHg</td>
<td>82 ± 4.8</td>
<td>74 ± 5.6</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>57 ± 4.6</td>
<td>51.1 ± 5.5</td>
</tr>
<tr>
<td>dp/dt</td>
<td>9.72 ± 1.8</td>
<td>16.6 ± 3.4</td>
</tr>
<tr>
<td>−dp/dt</td>
<td>1,254 ± 185</td>
<td>1,195 ± 122</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>0.59 ± 0.03</td>
<td>0.27 ± 0.04*</td>
</tr>
<tr>
<td>τ, ms</td>
<td>39 ± 2</td>
<td>57 ± 5</td>
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GLP-1 did not improve post-ROSC LV function. Hemodynamic and LV function measurements are shown in Table 2. After cardiac arrest and resuscitation, there were significant decreases in LV function. Ejection fraction in both groups was decreased from baseline at all post-ROSC time points (P < 0.05). Although GLP-1-treated animals had statistically better rate of decrease of LV pressure (−dP/dt) at 1 and 4 h post-ROSC, and better relaxation τ at 1 h, the treatment groups were comparable at other time points. Post-ROSC aortic systolic and diastolic pressures declined from baseline in the NS group, but this was not observed in the GLP-1 group (P < 0.05).

**Results**

**Table 1.** Resuscitation variables were similar between treatment groups.

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Values are means ± SE; n = 5 for normal saline (NS) and glucagon-like peptide-1 (GLP-1).

Post-ROSC GLP-1 administration preserved coronary microvascular endothelial function. CFR was measured by using the endothelium-dependent vasodilator Substance P. Animals treated with GLP-1 after ROSC did not show any significant decline in CFR compared with the pre-arrest measurements, nor did their mean CFR values ever fall below baseline measurements. In contrast, there was a significant decline in CFR in the control (NS) group from the pre-arrest baseline at both 1 and 4 h after ROSC (Fig. 1). This post-ROSC decline reflects coronary microvascular endothelial dysfunction in the control group, which was not observed in the GLP-1 group (P < 0.05).

**GLP-1 treatment did not affect whole blood glucose or plasma insulin concentrations.** GLP-1 is a potentiator of glucose-induced insulin secretion, and insulin has vasculo- and cardio-protective properties. In the present study, no change in glucose or insulin concentration from baseline was observed in either group (Table 3). In addition, no significant difference was found in either plasma insulin or blood glucose concentrations between groups at any timepoint.

**GLP-1 treatment decreased oxidant injury as measured by plasma 8-iso-PGF_{2α}**. In plasma drawn from the coronary sinus 4 h after ROSC, 8-iso-PGF_{2α} was significantly elevated in the control (NS) group, compared with the GLP-1 treatment group.
GLP-1 PREVENTS CORONARY MICROVASCULAR ENDOTHELIAL DYSFUNCTION

Fig. 1. Glucagon-like peptide-1 (GLP-1) improves coronary microvascular endothelial function after cardiac arrest and resuscitation. Coronary flow reserve (CFR) was measured using the endothelium-dependent vasodilator substance P. When compared with baseline measurements for each animal, GLP-1 treatment after return of spontaneous circulation (ROSC) prevented coronary microvascular endothelial dysfunction. *$P < 0.05$, and 4 h vs. baseline, same group; †$P < 0.05$, NS vs. GLP-1-treated animals; $n = 5$ animals each group.

**GLP-1 treatment did not affect SOD activity.** No difference in SOD activity was found in plasma from either the systemic circulation or the coronary sinus 4 h after ROSC (Table 3).

**GLP-1 treatment did not effect COX-2 protein expression.** Synthesis of 8-iso-PGF$_{2\alpha}$ from arachidonic acid can be synthesized nonenzymatically from oxygen free-radicals or enzymatically by COX-2. There was no difference in the expression of COX-2 protein in myocardium (Fig. 2) or aorta (data not shown) between groups 24 h after ROSC.

**DISCUSSION**

GLP-1 prevents coronary microvascular endothelial dysfunction after cardiac arrest and resuscitation, a finding that supports our hypothesis. Moreover, in plasma from the coronary sinus obtained 4 h after ROSC, GLP-1 treatment was associated with a decreased concentration of 8-iso-PGF$_{2\alpha}$, suggesting an antioxidant effect of GLP-1 in the coronary microcirculation. Contrary to our hypothesis, GLP-1 treatment did not improve LV function after cardiac arrest and resuscitation.

**Coronary microvascular dysfunction is a major contributor to poor outcomes after cardiac arrest and resuscitation (76).** Postresuscitation dysfunction of the microcirculation is associated with increased morbidity and mortality (35, 93) and is not prevented by current medical care (31).

Previous studies from our group (28, 57, 58) demonstrate a predictable decline in coronary microvascular function after cardiac arrest and resuscitation. Using the technique of in vivo CFR measurements in swine, Kern et al. (57, 58) found that as early as 30 min after resuscitation from cardiac arrest, CFR declined to 50% of baseline, and this dysfunction persisted for the duration of the 4-h follow-up period (57, 58). This experimental model also mimics the clinical manifestation of reperfusion injury after intervention for acute coronary syndromes, where epicardial coronary blood flow is restored but tissue perfusion continues to be compromised due to lack of blood flow through smaller vessels (85, 99). After myocardial infarction followed by reperfusion therapy, 30% of patients have dysfunction of the coronary microcirculation (99), which predicts cardiovascular morbidity (46, 73, 90, 105) and mortality.

**Table 3. Plasma insulin, SOD activity, and 8-iso-PGF$_{2\alpha}$ and whole blood glucose**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>30 Min</th>
<th>1</th>
<th>2</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>92 ± 8</td>
<td>143 ± 18*</td>
<td>128 ± 17*</td>
<td>112 ± 7.7</td>
<td>109 ± 9.0</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>3.20 ± 0.002</td>
<td>N/A</td>
<td>N/A</td>
<td>3.21 ± 0.001</td>
<td></td>
</tr>
<tr>
<td>8-IsopGF$_{2\alpha}$, pg/ml</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>38.07 ± 1.54</td>
<td></td>
</tr>
<tr>
<td>SOD activity, units/ml</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>0.33 ± 0.2</td>
<td></td>
</tr>
<tr>
<td><strong>GLP-1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>84 ± 6</td>
<td>177 ± 31*</td>
<td>140 ± 24*</td>
<td>120 ± 15.4</td>
<td>102 ± 11.0</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>3.21 ± 0.001</td>
<td>N/A</td>
<td>N/A</td>
<td>3.21 ± 0.002</td>
<td></td>
</tr>
<tr>
<td>8-IsopGF$_{2\alpha}$, pg/ml</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>29.59 ± 1.6*</td>
<td></td>
</tr>
<tr>
<td>SOD activity, units/ml</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>0.30 ± 0.2</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 5$ for all groups. N/A, not applicable. *$P < 0.05$, each time point compared with baseline measurements.
(12, 48, 73, 99). In fact, after myocardial infarction, decreased flow through the coronary microcirculation is a more powerful predictor of poor cardiovascular outcome (105) and mortality (48) than infarct size. Thus reperfusion-induced coronary microvascular dysfunction is a major health issue.

Beneficial effects of GLP-1 on the vasculature have been reported by others. GLP-1 improves endothelial function in vivo (4, 77, 78), decreases inflammatory injury in intact endothelium (32), attenuates the expression of pro-inflammatory cytokines (66) and adhesion molecules (65) in cultured endothelial cells, and preserves coronary microvascular function after cardiac arrest and resuscitation (28). In addition, GLP-1 protects the heart from reperfusion injury after myocardial infarction in a variety of settings (3, 13–15, 30, 50, 86, 98, 101).

The present study demonstrated no benefit of GLP-1 on systolic function and only a trend toward improvement in diastolic function. These findings are paradoxical, given the prognostic relationship between CFR and LV function (46–48, 53, 73, 90). Inadequate perfusion of the myocardium via the microcirculation is associated with larger myocardial infarcts, greater impairment of LV function, and a worse clinical outcome than normal microvascular perfusion (45).

The contractile benefits of preserving coronary microvascular function during reperfusion may be imperceptible during the acute period and become apparent long after the ischemic injury. Using myocardial contrast echocardiography, Ragosta and colleagues (84) determined that after acute myocardial infarction, up to 4 wk were required to exhibit improved LV function in regions of the myocardium exhibiting a functional microcirculation compared with regions with decreased microvascular perfusion (84). Araszkiewicz et al. (2) reported an association between sufficient microvascular reperfusion and improved LV function up to 6 mo after angioplasty in patients with acute myocardial infarction (2). In addition, LV function progressively improved over a 6-mo follow-up period in patients with normal coronary microvascular function after myocardial infarction versus those with poor microvascular function (12). Clearly, after reperfusion, a functional coronary microcirculation decreases the risk of LV dysfunction. A direct causal relationship and the time course involved are, however, unclear.

In contrast with findings from the present study, several groups have reported significant improvements in LV function with GLP-1 treatment; however, these studies were conducted in models other than cardiac arrest and resuscitation, which causes more profound, global myocardial stunning compared with regional contractile dysfunction after myocardial infarction (55). In isolated hearts, both GLP-1 (108) and exendin-4, a GLP-1 receptor agonist (98), improved myocardial contractility after ischemia. Poornima et al. (81) reported improvements in LV function and survival after GLP-1 treatment in rats prone to heart failure. In addition, treatment with GLP-1 improved LV performance in canine models of acute myocardial infarction (74) and dilated cardiomyopathy (75). In humans, GLP-1 improved ejection fraction in chronic heart failure (97) and prevented myocardial stunning after coronary balloon occlusion (87) and in the setting of ischemia in patients with coronary artery disease (88).

Although the etiology of postresuscitation coronary microvascular endothelial dysfunction is multi-factorial, oxidant injury is a major contributor (51) and correlates with poor outcomes (5, 51). In addition, antioxidant treatment improves postresuscitation recovery in the heart (96) and brain (7, 40). After cardiac arrest, the majority of ROS are produced 15–60 min postresuscitation (40). Oxidant injury is difficult to quantify, but the plasma concentration of 8-iso-PGF2α has emerged as a valid biomarker of oxidant stress in the CV system (63, 69), particularly after coronary ischemia and reperfusion (6, 27, 44, 68, 89, 92). Synthesis of 8-iso-PGF2α may be mediated by the enzyme COX-2 (59), but under conditions of oxidant stress, nonenzymatic synthesis by free radicals is more likely to occur (60, 106). In the present study, neither COX-2 expression nor SOD activity were different between treatment groups, suggesting that GLP-1 decreased synthesis of 8-iso-PGF2α via nonenzymatic mechanisms.

To our knowledge, the effects of GLP-1 as a direct antioxidant have not been tested in vitro. However, our findings of decreased 8-iso-PGF2α production in the coronary microcirculation support the idea that GLP-1, directly or indirectly, has antioxidant properties, particularly after ischemia and reperfusion. Consistent with our findings, others reports that GLP-1 decreases oxidant stress in a variety of tissues (3, 18, 21, 79, 83, 100, 103) and specifically mitigates endothelial production of reactive oxygen species (11). A limitation of the present study is the lack of identification of the source of ROS generation; however, we suspect the majority of ROS produced in the coronary microcirculation was mediated by NADPH oxidase, the primary source of ROS in vascular tissues (39, 42). In a study conducted by Loukogeorgakis et al. (67), endothelial dysfunction was prevented in humans lacking functional NADPH oxidase who were subjected to upper limb ischemia followed by reperfusion, compared with control subjects who exhibited a reduction in blood flow under the same conditions (67). These findings indicate a significant effect of NADPH oxidase in the endothelium. In the heart, formation of 8-iso-PGF2α, a lipid peroxidation product of ROS, is restricted to endothelial cells (68, 107) and vascular smooth muscle cells (91), and is not produced in cardiomyocytes (68). The activity of NADPH oxidase and formation of 8-iso-PGF2α is inversely proportional in human platelets (19, 80) and vascular tissues from hypertensive rats (82). Thus the most likely source of the majority of ROS formation in the coronary microcirculation is NADPH oxidase.

In addition to its role as a marker of oxidant stress, 8-iso-PGF2α is also a potent vasoconstrictor in a variety of vascular beds (24, 26, 38, 49, 52, 94, 102). Therefore, enhanced postresuscitation microvascular function in the heart by GLP-1 could be related to improved responses to endogenous vasodilating signals, as well as to decreased production of endogenous vasoconstricting agents, or both. The mechanisms behind ROS production in this model, and the antioxidant and vasoprotective effects of GLP-1, remain unclear. Compelling evidence will require the addition of a group of GLP-1-treated animals in which the antioxidant effect is blocked, which would provide excellent support for the causal relationship of GLP-1 in the preservation of coronary microvascular function, as well as evidence of an antioxidant mechanism.

An additional limitation of this study is the use of Substance P as the endothelium-dependent vasodilator in the measurements of CFR. Our group based this decision on previous success with this agent (unpublished observations); however, in hindsight, the more widely used and endothelium-specific vasodilator bradykinin (33) would have been optimal and...
allowed for greater comparison of our results with other studies. Moreover, healthy, juvenile swine were used in this study. Although swine are excellent animal models for the human cardiovascular system, cardiac arrest typically occurs in older humans with cardiovascular risk factors (such as diabetes) and pre-existing heart disease (72). The present study would be more relevant to the human condition had we used older swine with metabolic abnormalities and atherosclerosis. Thus these findings cannot be generalized to the typical cardiac arrest population.

In conclusion, continuous intravenous infusion of GLP-1 for 4 h after cardiac arrest and resuscitation preserved coronary microvascular endothelial function relative to saline placebo. These effects were associated with decreased 8-iso-PGF₂α production in the heart, indicating a possible antioxidant effect. GLP-1-based therapies are safe in humans and are currently in use for glycemic control in type 2 diabetes. Based on our findings, GLP-1 may be an effective treatment to preserve coronary microvascular function caused by reperfusion after ischemic events in the heart, and thus improve outcomes in this significant patient population.

GRANTS
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DISCLOSURES
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AUTHOR CONTRIBUTIONS
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REFERENCES
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