Glucagon-like peptide-1 preserves coronary microvascular endothelial function after cardiac arrest and resuscitation: Potential anti-oxidant effects.

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Hilwig, Kern, experimental design and procedures, manuscript review/edit.

Running Head: GLP-1 prevents coronary microvascular endothelial dysfunction

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Glucagon-like peptide-1 (GLP-1) has protective effects in the heart. We hypothesized that GLP-1 would mitigate coronary microvascular and left ventricular (LV) dysfunction if administered after cardiac arrest and resuscitation (CAR). 18 swine were subjected to ventricular fibrillation (VF) followed by resuscitation. Swine surviving to return of spontaneous circulation (ROSC) were randomized to receive an intravenous (IV) infusion of either human rGLP-1 (10 pmol/Kg/min, n=8) or 0.9% saline (n=8) for 4 hr, beginning 1 min after ROSC. CAR caused a decline in CFR in control animals (pre-arrest 1.86 ± 0.20, 1 hr post-ROSC 1.3 ± 0.05, 4 hr post-ROSC 1.25 ± 0.06, p<0.05). GLP-1 preserved CFR for up to 4 hr after ROSC (pre-arrest 1.31 ± 0.17, 1 hr post-ROSC 1.5 ± 0.01, 4 hr post-ROSC 1.55 ± 0.22). Although there was a trend toward improvement in LV relaxation in the GLP-1 treated animals, overall LV function was not consistently different between groups.

8-iso-PGF$_{2\alpha}$, a measure of reactive oxygen species (ROS) load, was decreased in post-ROSC GLP-1-treated animals (NS: 38.1 ± 1.54 pg/ml; GLP-1: 26.59 ± 1.56 pg/ml, p<0.05). Infusion of GLP-1 after CAR preserved coronary microvascular and LV diastolic function. These effects may be mediated through a reduction in oxidative stress.

Keywords: Swine, incretin, microcirculation, endothelium, coronary flow reserve

Although methods of resuscitation for victims of sudden cardiac arrest have improved, post-resuscitation complications still claim the lives of up to 60% of successfully resuscitated patients (61, 95). Cardiac arrest causes global ischemia, followed by marked reperfusion injury during and after successful resuscitation (1).

Reperfusion injury following cardiac arrest and resuscitation is significantly more severe than that caused by an ischemic event affecting a single organ or tissue (76). Inadequate tissue perfusion persists even after return of spontaneous circulation (ROSC), due to microcirculatory failure (31, 37) caused by endothelial injury (35, 76). Adequate blood flow through the microcirculation during and after resuscitation is predictive of survival (36). Further, persistent microvascular dysfunction is associated with organ failure and death (35, 93). Aside from therapeutic hypothermia (25), no clinical treatments for global reperfusion injury are available for use in clinical practice.

Amplified production of reactive oxygen species (ROS) occurs rapidly during reperfusion after cardiac arrest (41, 51), and is a leading cause of microvascular dysfunction (8). As early as 5 min after reperfusion, increased products of lipid peroxidation, biomarkers of free radical damage (6, 27, 63, 70), are present in the circulation (6, 8). Scavenging by antioxidants prior to reperfusion markedly decreases ROS production and improves blood flow through the microcirculation (8, 40). Isoprostanes are products of lipid peroxidation, and are biosynthesized in vivo.
through the free radical catalyzed oxidation of arachidonic acid (70). Specifically, 8-iso-
PGF$_{2\alpha}$ (8-epi- Prostaglandin F$_{2\alpha}$ isoprostane) has been well-characterized as a marker of oxidant stress after coronary ischemia and reperfusion (27, 62, 89).

Glucagon-like peptide-1 (GLP-1) is an endogenous incretin hormone with pleiotropic metabolic effects. GLP-1-related therapies are currently approved for the treatment of type 2 diabetes, and are believed to have protective effects on the pancreatic β-cell mass (17, 22, 34, 54, 104), although this has yet to be proven in humans. In addition, GLP-1 has beneficial effects in the heart (3, 13-16, 29) and the vasculature (4, 20, 28, 64). Perhaps most intriguing, Best et al found, in a retrospective analysis, a decreased incidence of cardiovascular events in diabetic patients taking a GLP-1 receptor agonist, when compared to diabetic patients taking other therapeutic agents for glycemic control (9).

We hypothesized that GLP-1 could mitigate post-resuscitation reperfusion injury and myocardial dysfunction. In addition, we hypothesized that GLP-1 would improve coronary microvascular endothelial function by decreasing oxidative stress. In an earlier study, we found that post-resuscitation treatment with GLP-1 improved coronary microvascular function as evaluated by adenosine-stimulated coronary flow reserve (CFR) (28). Because adenosine acts directly on vascular smooth muscle function (10) our previous experiments did not determine effects of GLP-1 on the coronary microvascular endothelium, the tissue most injured during reperfusion. The aim of the present study was to determine if administration of GLP-1 after cardiac arrest and resuscitation improves coronary microvascular or left ventricular (LV) function through effects on the coronary microvascular endothelium.

**METHODS**

**Animals and Procedures:**

This study was conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (23) after approval from the Institutional Animal Care and Use Committee of the University of Arizona. Domestic swine of either sex (30-35 Kg) were anesthetized and prepared for cardiac arrest and resuscitation as previously described (28, 55, 56, 58). Anesthesia was induced with 5% isoflurane inhalation anesthetic in oxygen administered by nose cone. An endotracheal tube was placed per os and a surgical plane of anesthesia was maintained 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 were adjusted to maintain end-tidal concentration of carbon dioxide (ETP$_{\text{CO}_2}$) at 40 ± 3 mmHg. Heart rate (HR), aortic pressure (AoP), electrocardiogram (ECG), end tidal partial pressure of carbon dioxide (ETP$_{\text{CO}_2}$) and tidal volume (TV) were continuously projected on a physiologic recorder (Ponemah Physiology Platform, Model P3 Plus, Gould Instrument Systems, Valley View, OH) for instantaneous visual monitoring and post-experimental 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 post-arrest using contrast left ventriculography. The time constant of isovolumetric LV relaxation (\( \tau \)) was obtained by using a high-fidelity, micromanometer-tipped pressure transducer (MPC-500, Millar Instruments, Houston, TX) placed in the LV. Cardiac output (CO), in L/min, was determined using a thermal dilution technique. Hemodynamic data were acquired (DAQ, Millar Instruments) and analyzed (Data Measuring Unit, MNPVS, 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 (LAD). 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 3 measurements was reported for each time point (baseline, 1 hr, and 4 hr after ROSC). Data were recorded for later calculation of coronary flow reserve (CFR) using the Flo-Map system (CardioMetrics Flo-Map, EndoSonics, Rancho Cordova, CA).

**Experimental Protocol and Treatments:**

Animals were blindly randomized to treatment or placebo (NS) group consisting of 9 animals each. Following the acquisition of pre-arrest baseline data and blood samples, ventricular fibrillation was electrically-induced by passing alternating current (60 cycles per second) through the electrodes of a catheter (locally made) in the right ventricle (RV) 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 (CPR) efforts, consisting of continuous chest compressions (CCC) at 100 per min and oxygen at 10 ventilator-administered breaths per min. One mg of epinephrine was given intravenously at the beginning of chest compressions. CPR was performed for 90 sec 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 mm Hg and a pulse pressure (aortic systolic minus diastolic pressure) of >20 mm Hg for 1 min. One minute after ROSC, an infusion pump (Alaris, Cardinal Health, Dublin, OH) was used to begin a 4 hr continuous infusion with either human rGLP-1 (American Peptide, 10 pmol/Kg/min) 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 hr after ROSC, final data were collected and animals were dis-instrumented (with the exception of one venous port), allowed to recover from anesthesia and placed in observation cages. Twenty-four hr post-ROSC a neurological function exam was conducted and the animals were humanely euthanized by IV 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, 1 hr, 2 hr and 4 hr after ROSC. Whole blood glucose (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 (1500 x g) for 5 minutes, 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, using a commercially available porcine insulin enzyme-linked immunoabsorbant assay (ELISA, Alpco Diagnostics, Salem, NH).

**Measurement of Plasma 8-iso-PGF2α and Superoxide Dismutase (SOD) Activity:**

Both systemic (drawn from carotid artery) and coronary sinus samples were used for these assays. Serum concentration of 8-iso-PGF2α was determined using a commercially-available enzyme-linked immunoabsorbant assay (EIA) kit (Cayman Chemical Co., Ann Arbor, MI). In serum, 8-iso-PGF2α may be esterified in lipids within the sample and will not be detected without hydrolysis. In order to obtain the total 8-isoprostane content (vs. free 8-iso-PGF2α fractions), serum samples were hydrolyzed with 15% potassium hydroxide followed by neutralization with a potassium phosphate buffer (pH 7.20). Subsequently, EIA for 8-iso-PGF2α 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 Co., Ann Arbor, MI), 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 nm and 450 nm for 8-iso-PGF2α and SOD activity, respectively.

**COX-2 Protein Expression:**

Twenty-four hr 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 left ventricle and aorta were immediately flash-frozen and stored at -80°C. Subsequently, tissues were separately homogenized in 8 vol of ice-cold lysis buffer (50 mM HEPES, 150 mM NaCl, 20 mM sodium pyrophosphate, 20 mM β-
glycerophosphate, 10 mM NaF, 2 mM Na₃VO₄, 2 mM EDTA, 1% Triton X-100, 10% glycerol, 1 mM MgCl₂, 1 mM CaCl₂, 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 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 (HRP; Everest Biotech). The proteins were visualized with an imaging system (Bio-Rad Chemi-Doc) after exposure to enhanced chemiluminescence detection reagents (Amersham Pharmacia, Piscataway, NJ). The band intensities on the images were quantified using Quantity One software (Bio-Rad).

**Data Analysis:** Data analyses were performed using commercially available software (SPSS, version 19.0, SPSS Inc., Chicago), and reviewed by a biostatistician. Prearrest haemodynamic measurements (5 time points) were compared between treatment groups using repeated-measures analysis of variance (ANOVA). CFR measurements were compared between treatment groups using a Wilcoxon rank-sum analysis to determine a change in post-ROSC measurements from pre-arrest measurements. Serum 8-iso-PGF₂α, SOD activity and protein expression (densitometry data) measurements were compared between groups using Student’s t-tests. Results were reported as mean ± SEM. A significant difference was reported if a p value < 0.05 was achieved.

**RESULTS**

**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 (PEA); three animals prior to treatment randomization, three NS animals and one GLP-1 animal during the 4 hr treatment period); or 2) expiration between 4 hr and 24 hr post-ROSC due to unknown causes (one GLP-1 animal was found expired during the monitoring period, 12 hr after ROSC). All 10 animals that completed the protocol and survived for 24 hr after ROSC were neurologically normal. Animals did not differ significantly in weight or gender 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 prior to achievement of ROSC (Table 1).

**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. EF in both groups was decreased from baseline at all post-ROSC time points ($p<0.05$). Although GLP-1 treated animals had statistically better $-\Delta P/\Delta t$ at 1 hr and 4 hr post-ROSC, and better relaxation $\tau$ at 1 hr, 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 ($p<0.05$).

Post-ROSC GLP-1 Administration Preserved Coronary Microvascular Endothelial Function.

Coronary flow reserve was measured 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 hr 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 Decreased Oxidant Injury as Measured by Plasma 8-iso-PGF$_{2\alpha}$.

In plasma drawn from the coronary sinus 4 hr after ROSC, 8-iso-PGF$_{2\alpha}$ was significantly elevated in the control (NS) group, compared with the GLP-1 treatment group ($p<0.05$, Table 3). No such increase was found in the pigs treated with GLP-1. In addition, no difference in 8-iso-PGF$_{2\alpha}$ was found between treatment groups in blood from the systemic circulation. Thus, there was a significant decrease in the production of 8-iso-PGF$_{2\alpha}$ in the coronary microcirculation of GLP-1-treated pigs during the first 4 hr of reperfusion after cardiac arrest.

GLP-1 Treatment did not Effect Superoxide Dismutase (SOD) Activity.

No difference in SOD activity was found in plasma from either the systemic circulation or the coronary sinus 4 hr after ROSC (Table 3).

GLP-1 Treatment did not Effect COX-2 Protein Expression.

Synthesis of 8-iso-PGF$_{2\alpha}$ from arachadonic acid can be synthesized non-enzymatically 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 hr 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 hr after ROSC, GLP-1 treatment was associated with a decreased concentration of 8-iso-PGF$_{2\alpha}$, suggesting an anti-oxidant 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). Post-resuscitation 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 coronary flow reserve (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 hr 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 (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 coronary flow reserve 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 MI, up to 4 weeks 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. reported an association between sufficient microvascular reperfusion and improved LV function up to 6 months after angioplasty in patients with acute MI (2). In addition, LV function progressively improved over a 6-month follow up period in patients with normal coronary microvascular function after MI vs. 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 to 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 to 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 post-resuscitation coronary microvascular endothelial dysfunction is multi-factorial, oxidant injury is a major contributor (51) and correlates with poor outcomes (5, 51). In addition, anti-oxidant treatment improves post-resuscitation recovery in the heart (96) and brain (7, 40). Following cardiac arrest, the majority of ROS are produced 15-60 min post-resuscitation (40). Oxidant injury is difficult to quantify, but the plasma concentration of 8-iso-PGF$_2\alpha$ 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-PGF$_2\alpha$ may be mediated by the enzyme COX-2 (59), but under conditions of oxidant stress, non-enzymatic 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-PGF$_2\alpha$ via non-enzymatic mechanisms.

To our knowledge, the effects of GLP-1 as a direct anti-oxidant have not been tested in vitro. However, our findings of decreased 8-iso-PGF$_2\alpha$ production in the coronary microcirculation support the idea that GLP-1, directly or indirectly, has anti-oxidant 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., endothelial dysfunction was prevented in humans lacking functional NADPH oxidase who were subjected to upper limb ischemia followed by reperfusion, compared to 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-PGF$_2\alpha$, 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-PGF$_2\alpha$ 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-PGF$_2\alpha$ is also a potent vasoconstrictor in a variety of vascular beds (24, 26, 38, 49, 52, 94, 102). Therefore, enhanced post-resuscitation 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 vasculoprotective 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 hr after cardiac arrest and resuscitation preserved coronary microvascular endothelial function relative to saline placebo. These effects were associated with decreased 8-iso-PGF$_2\alpha$ production in the heart, indicating a possible anti-oxidant 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.

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Table and Figure Legends:

Table 1. Resuscitation variables were similar between treatment groups.

Table 2. Hemodynamic variables, pre-arrest (baseline), 1 and 4 hr after ROSC. *P<0.05 compared to baseline measurements. ‡ p<0.05 compared to NS (placebo) group.

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

Figure 1. GLP-1 improves coronary microvascular endothelial function after cardiac arrest and resuscitation. CFR was measured using the endothelium-dependent vasodilator Substance P. Compared to baseline measurements for each animal, GLP-1 treatment after ROSC prevented coronary microvascular endothelial dysfunction.*P<0.05, 1 hr and 4 hr vs. baseline, same group. ‡P<0.05, NS vs. GLP-1 treated animals. N=5 animals each group.

Figure 2. No effect of GLP-1 treatment on COX-2 protein expression in myocardium or aorta of pigs after cardiac arrest and resuscitation. Pigs were subjected to VF and left untreated for 8 min. Beginning 1 min after ROSC, rGLP-1 (10 pmol/Kg/min) was continuously infused for 4 hrs. Following treatment period, pigs were monitored until 24 hr after ROSC. Immediately prior to euthanization, sections of aorta and myocardium were obtained and flash frozen. Subsequently, assessment of COX-2 protein expression was performed as described in METHODS. Representative immunoblot of myocardial homogenate from pigs in each treatment group is shown.
Table 1. *Resuscitation variables were similar between treatment groups.*

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<thead>
<tr>
<th>Resuscitation Variables</th>
<th>NS (n=5)</th>
<th>GLP-1 (n=5)</th>
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<tr>
<td>Shocks (mean # ± SEM)</td>
<td>1.2 ± 0.2</td>
<td>1.6 ± 0.2</td>
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<td>Epinephrine (mg ± SEM)</td>
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<td>8.0 ± 1.6</td>
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<td>Time in VF (sec ± SEM)</td>
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<td>577 ± 58</td>
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<td>HR (bpm ± SEM)</td>
<td>119 ± 8.7</td>
<td>132 ± 2.1</td>
</tr>
<tr>
<td>CO (L/min ± SEM)</td>
<td>2.8 ± 0.9</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td>AoS (mmHg ± SEM)</td>
<td>80 ± 2.1</td>
<td>72 ± 3.6</td>
</tr>
<tr>
<td>AoD (mmHg ± SEM)</td>
<td>53 ± 0.6</td>
<td>44.6 ± 3.2*</td>
</tr>
<tr>
<td>LVEDP (mmHg ± SEM)</td>
<td>9.2 ± 0.14</td>
<td>10.9 ± 1.0</td>
</tr>
<tr>
<td>dP/dt (± SEM)</td>
<td>1335 ± 68</td>
<td>1234 ± 140</td>
</tr>
<tr>
<td>-dP/dt (± SEM)</td>
<td>969 ± 88</td>
<td>656 ± 87</td>
</tr>
<tr>
<td>LVEF (% ± SEM)</td>
<td>0.57 ± 0.03</td>
<td>0.31 ± 0.01*</td>
</tr>
<tr>
<td>τ, (ms ± SEM)</td>
<td>45 ± 4</td>
<td>59.3 ± 4.2*</td>
</tr>
<tr>
<td></td>
<td>GLP-1</td>
<td>Baseline (n=5)</td>
</tr>
<tr>
<td>HR (bpm ± SEM)</td>
<td>109 ± 1.2</td>
<td>140 ± 9.3</td>
</tr>
<tr>
<td>CO (L/min ± SEM)</td>
<td>2.7 ± 0.2</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>AoS (mmHg ± SEM)</td>
<td>82 ± 4.8</td>
<td>74 ± 5.6</td>
</tr>
<tr>
<td>AoD (mmHg ± SEM)</td>
<td>57 ± 4.6</td>
<td>51.1 ± 5.5</td>
</tr>
<tr>
<td>LVEDP (mmHg ± SEM)</td>
<td>9.72 ± 1.8</td>
<td>16.6 ± 3.4</td>
</tr>
<tr>
<td>dP/dt (± SEM)</td>
<td>1254 ± 185</td>
<td>1195 ± 122</td>
</tr>
<tr>
<td>-dP/dt (± SEM)</td>
<td>1001 ± 95</td>
<td>671 ± 109*</td>
</tr>
<tr>
<td>LVEF (% ± SEM)</td>
<td>0.59 ± 0.03</td>
<td>0.27 ± 0.04*</td>
</tr>
<tr>
<td>τ, (ms ± SEM)</td>
<td>39 ± 2</td>
<td>57 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SEM. HR, heart rate; CO, cardiac output; AoS, aortic systolic pressure; AoD, aortic diastolic pressure; LVEDP, left ventricular end diastolic pressure; dP/dt, rate of increase of left ventricular pressure; -dP/dt, rate of decrease of left ventricular pressure; τ, isovolumetric relaxation time. *P<0.05, each timepoint compared to baseline measurements. ‡P<0.05 GLP-1 treatment compared to NS (placebo).
Table 3. Plasma insulin, SOD activity and 8-iso-PGF$_2\alpha$ and whole blood glucose.

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n=5)</th>
<th>30 Min (n=5)</th>
<th>1 Hr (n=5)</th>
<th>2 Hr (n=5)</th>
<th>4 Hr (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NS (n=5)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dl ± SEM)</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 ± SEM)</td>
<td>3.20 ± 0.002</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>3.21 ± 0.001</td>
</tr>
<tr>
<td>8-iso-PGF$_2\alpha$ (pg/ml ± SEM)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>38.07 ± 1.54</td>
</tr>
<tr>
<td>SOD activity (U/ml)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>0.33 ± 0.2</td>
</tr>
<tr>
<td><strong>GLP-1 (n=5)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dl ± SEM)</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 ± SEM)</td>
<td>3.21 ± 0.001</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>3.21 ± 0.002</td>
</tr>
<tr>
<td>8-iso-PGF$_2\alpha$ (pg/ml ± SEM)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>29.59 ± 1.6*</td>
</tr>
<tr>
<td>SOD activity (U/ml)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>0.30 ± 0.2</td>
</tr>
</tbody>
</table>
Figure 1.

![Graph showing CFR (normalized to baseline)](image)

- **NS**
- **GLP-1**

*Indicates statistical significance.
Figure 2.

[Image of gel electrophoresis showing COX-2 and GLP-1 treatments with molecular weight markers in kilodaltons (kDa)].