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Single Dose GLP-1-Tf Ameliorates Myocardial Ischemia/ Reperfusion Injury

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Abstract

Background—Glucagon-like peptide-1 (GLP-1) has insulinomimetic, insulinotropic and antiapoptotic properties that may make it a useful adjunct to reperfusion therapy for myocardial infarction (MI); however, GLP-1 has a short plasma half-life. Fusion of GLP-1 to human transferrin (GLP-1-Tf) significantly prolongs drug half-life.

Materials & Methods—We tested the ability of single dose GLP-1-Tf to limit myocardial ischemia (30 minutes) /reperfusion (180 minutes) injury in rabbits. Nineteen animals were untreated controls. The pre-ischemic group (n=10) was given 10mg/kg of GLP-1-Tf 12 hours before ischemia. Immediately after reperfusion, the post-ischemic group (n=10) received GLP-1-Tf (10mg/kg) and the Tf group (n=4) received transferrin alone.

Results—Infarct size as a percentage of the area at risk was $59.1 \pm 1.3\%$, $45.7 \pm 1.9\%$, $44.1 \pm 3.3\%$, $59.7 \pm 2.0\%$ in the control group, pre-ischemic group, post-ischemic group and Tf group, respectively ($p < 0.05$ for both GLP-1-Tf treatments group vs. control). GLP-1-Tf reduced the apoptotic index from $4.67 \pm 0.40\%$ in the control group to $3.15 \pm 0.46\%$ in the pre-ischemic group and to $2.66 \pm 0.40\%$ in the post-ischemic group ($p < 0.05$ for both GLP-1-Tf treatments vs. control). The size of the wall motion abnormality and ejection fraction was significantly improved in the post-ischemic group relative to the control group. Serum GLP-1 levels were $239.8 \pm 25.7 \mu\text{g/ml}$ in the post-ischemic group, $27.9 \pm 5.8 \mu\text{g/ml}$ in the pre-ischemic group and undetectable in the control group.

Conclusion—GLP-1-Tf limits myocardial reperfusion injury whether given prior to the onset of ischemia or given at reperfusion. GLP-1-Tf may also limit myocardial stunning at high serum levels of the drug.

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Keywords

Glucagon-like-peptide-1; myocardial infarction; myocardial reperfusion injury; post-ischemic conditioning; apoptosis

Introduction

Glucagon-like peptide-1 (GLP-1) is a member of the pro-glucagon incretin family implicated in the control of appetite and satiety [1]. This peptide has a unique constellation of biologic properties that may make it an effective adjunct to reperfusion therapy for acute myocardial infarction (AMI). GLP-1 has insulinomimetic [2] and insulinotropic [3] actions as well as antiapoptotic properties [4,5].

Insulin administered with glucose and potassium (GIK) has been demonstrated to have salutary metabolic and direct cardioprotective properties for ischemic myocardium [6,7]. Activation of the GLP-1 receptor in many cell types, including cardiac myocytes, has been shown to have important antiapoptotic properties that are independent of its insulin-potentiating effects and result from up-regulation of cyclic adenosine monophosphate (cAMP), as well as increased activity of phosphoinositide 3-kinase (PI3K), which is a central component of the reperfusion injury salvage kinase (RISK) pathway [8,9,10,11].

Reperfusion therapy using either pharmacologic or mechanical means has been established as an effective strategy for improving early and late outcomes in patients suffering AMI. Reperfusion, however, is always responsible for some degree of myocyte loss [12] with apoptosis being increasingly recognized as an important contributor to reperfusion-induced myocardial injury [13]. The insulin potentiating and antiapoptotic properties of GLP-1 make it a potentially attractive adjunct to reperfusion therapy for AMI. The effects of GLP-1 are predicated on ambient glucose concentration and are mitigated at plasma glucose concentrations <70 mg/dl, which minimizes the risk of hypoglycemia and the need for concomitant glucose infusion, further increasing the compound's clinical potential [14].

Because of rapid enzymatic inactivation by dipeptidyl peptidase-IV (DPPIV), GLP-1 has a very short circulating half-life (1–2 minutes) that requires a continuous infusion for therapeutic levels of the peptide to be maintained [11]. A fusion protein has been developed which consists of a DPPIV-resistant GLP-1 analog fused to non-glycosylated human transferrin (GLP-1-Tf). This fusion protein has a much longer half-life (27 hours in rabbits), while retaining its ability to directly activate GLP-1 receptors.

We studied the effect of single dose GLP-1-Tf on myocardial reperfusion injury (infarct size and degree of myocyte apoptosis) as well as regional and global left ventricular function when administered either before myocardial ischemia or at the time of reperfusion in an *in vivo* rabbit model of myocardial ischemia/reperfusion.

Materials and Methods

Preparation of GLP-1-Tf

GLP-1-Tf was made by fusing the DNA sequence of GLP-1 (7–37) to the N-terminus of human transferrin DNA through a linker DNA sequence. To increase stability during expression, the lysine at position 34 of GLP-1 was modified to alanine (K34A) to inhibit inactivation by neutral endoprotease, and to prevent dipeptidyl-peptidase cleavage of the GLP-1 moiety *in vivo*, the alanine at position 8 was changed to glycine (A8G). The human transferrin sequence was also altered at two positions to prevent N-linked glycosylation of

the protein. The fusion protein was cloned and expressed in the yeast *Saccharomyces cerevisiae* and the expressed protein was purified from the fermentation supernatant. The fusion protein specifically activates the GLP-1 receptor.

Surgical Protocol

Forty-three New Zealand White rabbits (2.9–4.6kg) were utilized in four experimental groups (Figure 1):

1. Control (n=19): sham saline injection
2. Pre-Ischemic Treatment Group (Pre-GLP-1-Tf) (n=10): single dose GLP-1-Tf (10mg/kg) given subcutaneously 12 hours prior to ischemia
3. Post-Ischemic Treatment Group (Post-GLP-1-Tf) (n=10) single dose GLP-1-Tf (10mg/kg) given intravenously at the onset of reperfusion.
4. Post-Ischemic Transferrin Group (Tf) (n=4): Single dose of Tf (10mg/kg) given intravenously at the onset of reperfusion

Animals were treated under experimental protocols approved by the University of Pennsylvania's Institutional Animal Care and Use Committee (IACUC). The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996). Anesthesia was induced with intramuscular ketamine (70–100mg/kg), glycopyrrolate (0.01mg/kg) and buprenorphine (0.05mg/kg). After oral endotracheal intubation, animals were mechanically ventilated (Hallowell EMC Model AWS, Pittsfield, MA, USA) with air enriched with 0.6 L/min of oxygen. Anesthesia was maintained with continuous ketamine infusion (20mg/kg/hr IV). A high fidelity pressure transducer (Millar Instruments Inc. Houston, TX, USA) was placed in the left ventricle (LV) via carotid artery for continuous LV pressure measurement. Peripheral arterial blood pressure, heart rate and surface electrocardiogram (ECG) were also continuously monitored (Hewlett Packard 78534C, Palo Alto, CA, USA) and recorded (Sonometrics Inc., London, Ontario, Canada). Left atrial blood temperature was measured with an electrical thermometer (Thermalert TH-8 Physiotemp Instrument, Clifton, NJ, USA) and was maintained between 39–40°C (normal rabbit body temperature) with a high efficiency water blanket (Medi-Therm III, Gaymar Industries Inc., Orchard Park, NY, USA). A left thoracotomy was performed in the fourth intercostal space and the heart was exposed. A pledgetted suture (3-0 Ti-cron, U.S. Surgical, Norwalk, CT, USA) was passed around a large branch of the circumflex coronary artery at a distance 50% from the base of the heart toward the apex to reliably obtain a risk area of 28 to 30% of the left ventricular mass. Myocardial ischemia was achieved by tightening the coronary artery snare and confirmed by ST elevations on ECG and by the distinct color change of the myocardium. After 30 minutes of ischemia, the coronary artery snare was released and the myocardium was reperfused for 180 minutes.

Hemodynamic Data

Heart rate, LV pressure, peripheral blood pressure and ECG were recorded after induction of anesthesia and placement of monitoring lines (baseline), fifteen minutes after coronary occlusion, ten minutes after reperfusion and three hours after reperfusion immediately before the animal was sacrificed. The rate-pressure product was calculated as measure of LV systolic function [15].

Risk Area and Infarct Size Measurement

Following completion of reperfusion, the coronary snare was re-tightened and vascular clamps were used to occlude the aorta, pulmonary artery and inferior vena cava, and the

right atrium was incised. Five milliliters of Evans blue dye (1%) (Sigma, St. Louis, MO, USA) was injected via the left atrium to delineate the ischemic myocardial risk area (RA). The heart was arrested with intra-atrial bolus of 20 mEq of potassium chloride and the heart was explanted. The left ventricle was sectioned perpendicular to its long axis into 6–7 slices (Figure 2). The thickness of each slice was measured with a digital micrometer and a standardized digital photograph was taken (Casio EX-Z850, Tokyo, Japan). Infarct area (IA) was delineated by photographing and measuring the slices after 20 minutes of incubation in 2% triphenyltetrazolium chloride (TTC) at 37° C. All photographs were imported into an image analysis program (Image Pro Plus, Media Cybernetics, Silver Spring, MD, USA) and computerized planimetry was performed. The RA is expressed as a percentage of the LV, and the infarct size is expressed as a percentage of the RA (IA/RA). The entire RA from each of the LV slices was excised and placed in formalin, dehydrated in ethanol, and wax embedded in paraffin.

Assessment of Apoptosis

The extent of apoptosis within the RA was assessed with *In Situ* Oligo Ligation (ISOL) assay (Intergen #7200; Serological Corp, Norcross, GA) with a high specificity for staining the specific DNA fragmentation characteristic. ISOL was used because it is the most specific of the *in situ* enzymatic reactions for assessing apoptosis in myocardial specimens [16]. This assay utilizes T4 DNA Ligase to bind synthetic biotinylated oligonucleotides to 3'-dT overhangs. Paraffin-embedded tissue was sectioned into 5-μm micron slices and deparaffinized by three changes of xylene, followed by three changes of absolute ethanol. Subsequently, endogenous peroxidase was quenched in 3% hydrogen peroxide in phosphate buffered saline (PBS). After washing the tissue sections, they were treated with 20 μg/mL of proteinase K in PBS, washed again, and placed in an equilibration buffer. Next, a solution of T4 DNA ligase and oligonucleotides was applied to the slides and incubated overnight at 16–22°C. ApopTag® Detection of ligated oligonucleotides was accomplished by applying a streptavidin-peroxidase conjugate that was developed with diaminobenzidine (DAB). Finally, tissue sections were counterstained in hematoxylin.

Entire tissue sections were digitalized using a scanning microscope and analyzed using an image analysis software package (Image Pro Plus, Media Cybernetics, Silver Spring, MD, USA). ISOL positive and ISOL negative nuclei were counted in the RA from both control and treatment by two investigators in a blinded fashion. Results are expressed as a percentage of ISOL positive cells/ total number of cells in the RA and referred to as the apoptotic index.

Measurement of GLP-1 Serum Levels

Blood samples were collected at 180 minutes after reperfusion and were centrifuged (5000 rpm × 4 minutes) with the serum retained and stored/archived at –80°C. These samples were analyzed for the serum levels of GLP-1. GLP-1 was captured from plasma on 96-well plates having mouse anti-GLP-1 monoclonal antibody bound to goat anti-mouse F(ab')₂. Bound GLP-1 was detected using biotinylated chicken anti-human transferrin, streptavidin-HRP, and QuantiBlu peroxidase fluorogenic substrate. Quantitation was done using a Gemini XPS fluorescent plate reader with excitation and emission wavelengths of 325 nm and 420 nm, respectively by comparison to a standard curve of GLP-1.

Echocardiography

Quantitative, two-dimensional, open-chest echocardiograms were performed at baseline, at 20 minutes of ischemia, at the onset of reperfusion and at 165 minutes of reperfusion in all animals. Images were obtained on a Phillips 7500 ultrasound system (Phillips Medical Systems, Andover, MA, USA) using a 12-MHz transducer (770020A, Hewlett-Packard)

with a custom-made offset device and recorded on 0.5-inch SVHS videotape at 30 Hz (Panasonic AG-6300 VHS Recorder). The transducer was placed at the cardiac apex and two orthogonal long-axis views were recorded. Left ventricular end-systolic volume (LVESV) and left ventricular end-diastolic volume (LVEDV) were measured with an off line analysis system (Tomtec Imaging Systems, Munich, Germany) using a modified Simpson's rule algorithm [17]. Ejection fraction (EF) was calculated as $[(LVEDV-LVESV)/LVEDV] \times 100$. The length of endocardial segment that was akinetic between diastole and systole and did not exhibit wall thickening was measured from the apical four-chambered view and reported as the wall motion abnormality (WMA).

Statistics

Results are expressed as the mean $\pm$ standard error of the mean. Repeated measures analysis of variance (ANOVA) was used to evaluate changes over time and to compare results between the groups. One way ANOVA with Bonferroni t-tests was used to detect differences between the groups at different time points. The software package used for the statistical analysis was SPSS version 11.0 for Windows (SPSS Inc, Chicago IL, USA).

Results

GLP-1 Levels

GLP-1 levels in the post-ischemic treatment group were $239.8 \pm 25.7 \mu\text{g/ml}$, which was significantly higher than in the pre-ischemic treatment group ($27.9 \pm 5.8 \mu\text{g/ml}$, $p < 0.0001$) and the control group in which levels of GLP-1 were undetectable ($< 0.009 \mu\text{g/ml}$, $p < 0.0001$). GLP-1 was administered subcutaneously to the pre-ischemic treatment group 12 hours prior to ischemia; clearance of the drug from the circulation and lower bioavailability via subcutaneous injection was expected to result in lower levels of the drug 180min post-reperfusion than in the post-ischemic treatment group that received the drug intravenously at the time of reperfusion.

Infarct Size and Myocyte Apoptosis

The RA was very similar in all groups and averaged between 28–30% of the LV mass. The IA as a percentage of the RA was $59.1 \pm 1.3\%$ in the control group. Administration of Tf alone did not affect infarct size ($59.7 \pm 2.0\%$). Relative to the control group, infarct size was significantly reduced in the pre-ischemic treatment group ($45.7 \pm 1.9\%$, $p < 0.0001$) and in the post-ischemic treatment group ($44.1 \pm 3.3\%$, $p < 0.0001$). Administration of GLP-1-Tf using either treatment strategy also significantly reduced the apoptotic index from $4.67 \pm 0.40\%$ in the control group to $3.15 \pm 0.46\%$ in the pre-ischemic treatment group ($p < 0.05$) and to $2.66 \pm 0.40\%$ in the post-ischemic treatment group ($p < 0.05$). These data are summarized in Table 1.

Echocardiographic Data

Echocardiographic data are summarized in Table 2. The ejection fraction (EF) at 165 minutes after reperfusion in the post-ischemic treatment group ($35.60 \pm 2.24\%$) was higher than control group ($29.73 \pm 1.24\%$, $p = 0.038$), Tf group ($30.25 \pm 1.65\%$, $p = 0.049$) and the pre-ischemic treatment group ($31.33 \pm 1.39\%$, $p = 0.19$) (Table 2, Figure 3). The length of the LV wall motion abnormality at 165 minutes after reperfusion was significantly reduced in the post-ischemic treatment group ($1.46 \pm 0.18\text{mm}$) compared to the control group ($1.89 \pm 0.09\text{mm}$, $p = 0.044$), Tf group ($1.89 \pm 0.07\text{mm}$, $p = 0.048$) and the pre-ischemic treatment group ($1.74 \pm 0.09\text{mm}$, $P = 0.05$). (Table 2, Figure 4)

Hemodynamics and Temperature Data

Temperature and hemodynamic data are summarized in Table 3. Core body temperatures were effectively maintained in a narrow range for all animals in all groups. There were no between group differences in hemodynamic data at any time point.

Discussion

Early and late mortality after AMI is significantly improved by expeditious mechanical or pharmacologic reperfusion of the myocardial risk area [18]. Extensive preclinical and clinical research has been focused on developing adjunctive cytoprotective pharmacological strategies to limit reperfusion injury and further increase the therapeutic benefit of re-establishing blood flow to the ischemic myocardial region. Ideally, such therapy would be safe, easily administered and effective when given at the time of reperfusion [19]. The data reported above suggests that GLP-1-Tf has these properties.

Native and recombinant forms of GLP-1 have a half-life of minutes, being rapidly degraded by DPPIV, to generate an NH₂-terminally truncated inactive metabolite in addition to undergoing renal excretion. Therefore, to be effective the GLP-1 peptide must be administered as a continuous infusion or given with an inhibitor of DPPIV as a means of reducing its degradation [20]. Fusion of GLP-1 to transferrin extends its plasma half-life to 27 hours in rabbits, obviating the need for continuous infusion or co-administration with agents to inhibit its degradation. In the current experiment, GLP-1-Tf maintained its cytoprotective effect for cardiac myocytes for over 12 hours after subcutaneous delivery.

We demonstrated that single dose GLP-1-Tf limits myocardial loss significantly and to the same extent whether given subcutaneously 12 hours prior to ischemia or intravenously at the time of reperfusion, indicating that the drug has its effect primarily by limiting reperfusion induced injury rather than by increasing myocardial tolerance for ischemia. This cardioprotective effect is due, at least in part, to a reduction in reperfusion induced myocyte apoptosis.

When measured 180 minutes post-reperfusion, serum GLP-1-Tf levels were 10 fold higher in the post-ischemic treatment group, compared to the pre-ischemic treatment group. Although this difference in drug concentration did not significantly influence myocardial salvage or apoptotic index, the extent of the wall motion abnormality was significantly less and the EF significantly higher in the post-ischemic treatment group than for the pre-ischemic treatment group. These data suggest that GLP-1-Tf at higher concentration in the reperfusion period adds further benefit by limiting non-lethal myocardial injury (i.e. myocardial stunning) and potentially acting as a positive inotrope.

Our results are consistent with the work of Bose and colleagues that demonstrated a cardioprotective effect of subcutaneously administered recombinant GLP-1 given in conjunction with valine pyrrolidide, a potent inhibitor of DPPIV, in both *in situ* and isolated rodent heart preparations [8]. Kavianipour and colleagues reported that a continuous infusion of recombinant GLP-1 reduced myocardial pyruvate and lactate accumulation in a porcine model of ischemia/reperfusion but did not reduce infarct size. These investigators did not employ an inhibitor of DPPIV so it is possible that GLP-1 may have been partly degraded, potentially limiting its cardioprotective properties. Alternatively, the length of ischemia in this experimental preparation may have overwhelmed the protective effect of the continuous infusion GLP-1 [21].

The mechanism for the cardioprotective properties of GLP-1 has not been fully elucidated; however our work and that of other investigators suggest that GLP-1 has important

antiapoptotic properties. An increasing body of experimental evidence supports the importance of myocyte apoptosis to myocardial reperfusion injury, identifying this cellular process as a valid therapeutic target for pharmacologic adjuncts to reperfusion therapy [13].

The GLP-1 receptor is widely expressed in islet cells, kidney, lung, brain, the gastrointestinal tract, and, interestingly, also in the heart [22]. GLP-1 has been shown to limit apoptosis in insulin secreting pancreatic β -cells and most recently in cardiac myocytes through cAMP and PI3K pathways via activation of the GLP-1 receptor [8,23]. The activation of PI3K is of particular interest since this kinase has been definitively associated with myocardial protection in the setting of ischemic/reperfusion injury [9] as well as myocardial preconditioning [24,25].

Insulin has a direct cardioprotective effect in animal models of ischemia/reperfusion that are distinct from its metabolic effects and are associated with a PI3K mediated reduction in myocyte apoptosis [7,26]. Extensive clinical interest has developed around the use of the “metabolic cocktail” of glucose, insulin and potassium (GIK) as an adjunct to reperfusion therapy for AMI. While clinical results have been variable and the logistics of administering continuous infusions of glucose and insulin in critically ill patients has been challenging, GIK appears to have beneficial effects when used as an adjunct to reperfusion therapy in at least some subsets of patients [27,28]. It is, therefore, possible that GLP-1, acting as a potent incretin, could increase levels of insulin, and its cardioprotective properties be explained on that basis. However, although a GLP-1 related rise in insulin and decrease in glucose is possible, the studies reported here were undertaken in fasted non-diabetic animals, suggesting that any GLP-1 mediated stimulation of insulin release was likely small because insulinotropic effects of GLP-1 are glucose dependent and are very limited at glucose levels below 70 mg/dl [14]. Furthermore, as discussed above, Bose and colleagues demonstrated similar degrees of myocardial protection in both intact animals and insulin-free isolated heart preparations which support an insulin independent mechanism for the myocardial protective effects of GLP-1 [8].

The association of GLP-1 with up-regulation of intracellular cAMP suggests that the drug may have positive inotropic properties. Experimental data with regard to the hemodynamic effects of GLP-1 are mixed. Yamamoto and colleagues have reported significant increases in blood pressure and heart rates in rats receiving GLP-1 [22], while Deacon and colleagues failed to confirm such hemodynamic effects in pigs [29]. Our echocardiographic data would suggest that GLP-1 acts as a positive inotrope when systemic levels are high. The discrepancy in previously published studies may be related to dosing differences and/or timing of the drug’s administration.

Clinically, Nikolaidis and colleagues reported that when added to standard therapy, continuous infusion of recombinant GLP-1 for three days improved regional and global LV function in patients with AMI and severe systolic dysfunction after successful primary angioplasty. Because of the experimental design of this study it was unclear whether the beneficial effect of GLP-1 was due to inotropic stimulation or whether it resulted from improved myocardial salvage; however, since these authors did not start administration of GLP-1 until 2 to 4 hours after reperfusion it is likely that the reported improvement in hemodynamic endpoints were attributable to changes in inotropic state rather than increased myocardial salvage [14].

In summary, GLP-1-Tf greatly increases the effective half-life of GLP-1 whether given subcutaneously or intravenously and limits myocardial injury in an in vivo rabbit model of ischemia/reperfusion, at least in part by reducing myocyte apoptosis. At higher doses, the drug may also limit myocardial stunning and act as a positive inotrope. Further studies will

be required to optimize dosage and timing, but this study supports the potential use of GLP-1-Tf as an adjunct to reperfusion therapy for AMI.

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Figure 1.
Schematic of the Experimental Design

Figure 2.

Representative photographs of rabbit left ventricles sectioned perpendicular to the long axis after staining with Evan's blue dye and triphenyltetrazolium chloride. A representative heart from the pre-ischemic treatment group is depicted in (A) and a representative heart from the control group is shown in (B). These figures show the area of infarction (pale pink), risk area (red and pale pink) and non-ischemic area (purple). Note the improved myocardial salvage in the pre-ischemic treated animal compared to control.

Figure 3.

The effects of GLP-1-Tf on ejection fraction as determined by serial, open-chest, quantitative, two-dimensional echocardiography. Measurements were taken at baseline (Base), at 20 minutes of ischemia (Isc-20), at the onset of reperfusion (Rep-0) and at 165 minutes of reperfusion (Rep-165) in all animals. (* $p < 0.05$ vs. control and Tf)

Figure 4.

The effects of GLP-1-Tf on the size of the wall motion abnormality as determined by serial, open-chest, quantitative, two-dimensional echocardiography. Measurements were taken at 20 minutes of ischemia (Isc-20), at the onset of reperfusion (Rep-0) and at 165 minutes of reperfusion (Rep-165) in all animals. (* $p < 0.05$ vs. control and Tf)

Table 1**Risk Area, Infarct Area & Apoptotic Index**

	RA/LV (%)	IA/LV (%)	IA/RA (%)	Apoptotic Index (%)
Control (n=19)	30.3 ± 1.4	17.8 ± 1.0	59.1 ± 1.3	4.67 ± 0.40
Pre-GLP-Tf (n=10)	29.2 ± 1.4	13.4 ± 0.9 ^{†,‡}	45.7 ± 1.9 ^{*,‡}	3.15 ± 0.46 ^{†,‡}
Post-GLP-Tf (n=10)	28.4 ± 3.0	12.6 ± 1.6 ^{†,‡}	44.2 ± 1.9 ^{*,‡}	2.66 ± 0.40 ^{†,‡}
Tf (n=4)	28.4 ± 1.9	17.0 ± 1.5	59.7 ± 2.0	4.33 ± 0.25

* Values are means ± SEM. Statistically significant difference from Control (p < 0.01) is indicated by

[†] statistically significant difference from Control (p < 0.05) is indicated by

[‡] statistically significant difference from Tf (p < 0.05) is indicated by

RA= risk area, IA= infarct area, LV= left ventricle.

Table 2

Echocardiographic Data

	Baseline	Ischemia 20 min	Reperfusion 0 min	Reperfusion 165 min
EF (%)				
Control	34.47 ± 1.00	30.25 ± 1.45	30.58 ± 1.72	29.73 ± 1.24
Pre-GLP-1-Tf	34.78 ± 3.25	30.75 ± 2.93	33.11 ± 1.64	31.33 ± 1.39
Post-GLP-1-Tf	34.60 ± 1.85	30.88 ± 1.94	33.50 ± 2.85	35.60 ± 2.24 ^{†,*}
Tf	33.75 ± 2.90	31.00 ± 0.91	31.75 ± 2.84	30.25 ± 1.65
WMA (cm)				
Control	-	2.23 ± 0.09	1.99 ± 0.07 [‡]	1.89 ± 0.09 [§]
Pre-GLP-1-Tf	-	2.22 ± 0.11	1.81 ± 0.07 [§]	1.74 ± 0.09 [‡]
Post-GLP-1-Tf	-	2.18 ± 0.17	1.69 ± 0.17 [‡]	1.46 ± 0.18 ^{‡,‡,*,#}
Tf	-	2.24 ± 0.08	2.06 ± 0.19	1.89 ± 0.07 [§]
SV (cm³)				
Control	1.22 ± 0.06	1.33 ± 0.11	1.30 ± 0.11	1.26 ± 0.08
Pre-GLP-1-Tf	1.25 ± 0.13	1.29 ± 0.14	1.40 ± 0.11	1.39 ± 0.07
Post-GLP-1-Tf	1.27 ± 0.08	1.25 ± 0.06	1.27 ± 0.10	1.32 ± 0.12
Tf	1.20 ± 0.20	1.33 ± 0.13	1.34 ± 0.19	1.30 ± 0.07

[†] Values are means ± SEM. Statistically significant difference from Control (p < 0.05) is indicated by

* statistically significant difference from Tf (p < 0.05) is indicated by

[‡] statistically significant difference from Ischemia 20 min (p < 0.01) is indicated by

[§] statistically significant difference from Ischemia 20 min (p < 0.05) is indicated by

[#] statistically significant difference from Reperfusion 0 min (p < 0.01) is indicated by

EF = Ejection Fraction; WMA = Wall Motion Abnormality; SV = Stroke Volume.

Table 3**Hemodynamics & Temperature Data**

	Baseline	Ischemia 15 min	Reperfusion 10 min	Reperfusion 180 min
HR (beats/min)				
Control	266 ± 4	258 ± 4	254 ± 4	248 ± 3 [§]
Pre-GLP-1-Tf	279 ± 14	249 ± 6	250 ± 7	255 ± 8
Post-GLP-1-Tf	260 ± 11	241 ± 9	242 ± 9	243 ± 8
Tf	276 ± 8	257 ± 6	257 ± 8	248 ± 5 [§]
MAP (mmHg)				
Control	65 ± 3	52 ± 2 [‡]	53 ± 2 [‡]	48 ± 2 [‡]
Pre-GLP-1-Tf	70 ± 4	45 ± 2 [‡]	47 ± 3 [‡]	49 ± 2 [§]
Post-GLP-1-Tf	72 ± 4	50 ± 3 [‡]	49 ± 3 [‡]	45 ± 1 [‡]
Tf	67 ± 5	53 ± 2 [§]	55 ± 2 [§]	53 ± 4 [§]
LVEDP (mmHg)				
Control	2.6 ± 0.7	5.9 ± 1.0 [‡]	5.1 ± 0.7 [‡]	4.9 ± 0.8 [‡]
Pre-GLP-1-Tf	2.1 ± 0.6	4.3 ± 0.6 [§]	3.8 ± 0.6	4.1 ± 0.6 [§]
Post-GLP-1-Tf	2.8 ± 0.8	4.0 ± 0.8	3.6 ± 0.6	4.0 ± 0.5
Tf	2.3 ± 0.2	5.7 ± 1.2	5.7 ± 1.2	4.4 ± 0.9
RPP / 1000 (mmHg/min)				
Control	20.5 ± 0.7	16.2 ± 0.5 [‡]	16.5 ± 0.7 [‡]	15.0 ± 0.5 [‡]
Pre-GLP-1-Tf	22.2 ± 1.8	13.8 ± 0.9 [‡]	13.9 ± 0.7 [§]	15.6 ± 1.0 [‡]
Post-GLP-1-Tf	21.4 ± 1.1	15.1 ± 1.1 [‡]	15.8 ± 1.1 [‡]	14.0 ± 0.4 [‡]
Tf	20.9 ± 1.0	16.5 ± 0.5 [‡]	16.7 ± 0.5 [‡]	15.7 ± 0.4 [‡]
Temperature (°C)				
Control	39.8 ± 0.1	39.6 ± 0.1	39.6 ± 0.1	39.7 ± 0.1
Pre-GLP-1-Tf	39.8 ± 0.2	39.4 ± 0.1	39.4 ± 0.0	39.6 ± 0.1
Post-GLP-1-Tf	39.6 ± 0.1	39.6 ± 0.1	39.4 ± 0.1	39.6 ± 0.1
Tf	39.7 ± 0.2	39.6 ± 0.1	39.5 ± 0.1	39.6 ± 0.1

[‡] Statistically significant difference from Baseline (p < 0.01) is indicated by

[§] statistically significant difference from Baseline (p < 0.05) is indicated by

Values are means ± SEM. HR = heart rate; MAP = mean arterial pressure; LVEDP = left ventricular end-diastolic pressure; RPP = rate pressure product; Temp = temperature in left atrium.