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The Direct Effects of Glucagon-like Peptide-1 (GLP-1) on Myocardial Contractility and Glucose Uptake in Normal and Post-Ischemic Isolated Rat Hearts

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Abbreviations: Glucagon-Like Peptide-1 (GLP-1); Rate-pressure product (RPP); Left ventricle (LV); Left ventricular end diastolic pressure (LVEDP)

Abstract

Background: Recent evidence suggests that GLP-1 enhances recovery of LV function following transient coronary artery occlusion. However, it is uncertain whether GLP-1 has direct effects on normal or ischemic myocardium and whether the mechanism involves increased myocardial glucose uptake. **Methods:** LV function and myocardial glucose uptake and lactate production were measured under basal conditions and following 30 minutes of low flow ischemia and 30 minutes of reperfusion in the presence and absence of GLP-1 (7-36) amide. The response was compared to standard buffer alone or buffer containing insulin (100μU/ml). **Results**: GLP-1 decreased the left ventricular developed pressure (Baseline: 100±2 mmHg; GLP-1: 75±3 mmHg, $p< 0.05$) and LV dP/dt (Baseline: 4,876±65 mmHg/sec; GLP-1: 4,353±76 mmHg/sec, $p< 0.05$) in normal hearts. GLP-1 increased myocardial glucose uptake (Baseline: 33±3 μmol/min/g; GLP-1: 81±7 μmol/min/g, $p< 0.05$) by increasing nitric oxide production and GLUT-1 translocation. GLP-1 enhanced recovery following 30 minutes of low flow ischemia with significant improvements in LVEDP (Con: 13±4 mmHg; GLP-1: 3±2 mmHg, $p< 0.05$) and LV Dev P (Con: 66±6 mmHg; GLP-1: 98±5 mmHg $p< 0.05$). GLP-1 increased L V function, myocardial glucose uptake and GLUT-1 and GLUT-4 translocation during reperfusion to an extent similar to insulin. **Conclusions:** GLP-1 has direct effects on the normal heart, reducing contractility, but increasing myocardial glucose uptake through a non-Akt-1 dependent mechanism, distinct from the actions of insulin. However, GLP-1 increased myocardial glucose uptake and enhanced recovery of cardiac function following low flow ischemia in a fashion similar to insulin.

Introduction

Glucagon –like peptide-1 (GLP-1) is a member of the pro-glucagon incretin family implicated in the control of appetite and satiety (Drucker, 1998; Doyle and Egan, 2001; Kieffer and Habener, 1999). GLP-1 has insulinotropic, insulinomimetic, and glucagonostatic effects, thereby exerting multiple complementary actions to lower blood glucose in subjects with type 2 diabetes mellitus (Drucker, 1998; Doyle and Egan, 2001; Kieffer and Habener, 1999). A major advantage over conventional insulin is the fact that the insulinotropic actions of GLP-1 are dependent on the ambient glucose concentration, mitigating the risks of hypoglycemia.

Although receptors for GLP-1 have been found in a variety of extra-pancreatic tissues including the heart (Bullock et al., 1996; Wei and Mojsov, 1996), whether there are direct cardiovascular actions of GLP-1 remains controversial. For example, GLP-1 increased heart rate and blood pressure in intact rodents through sympatho-stimulatory effects (Barragan et al., 1994; Barragan et al., 1996; Yamamoto et al., 2002), but depressed myocardial contractility in isolated rat ventricular myocytes (Vila Petroff et al., 2001). Recently, infusion of GLP-1 was associated with improvement in myocardial glucose uptake and LV systolic function in conscious dogs with dilated cardiomyopathy, but not in normal dogs (Nikolaidis et al., 2004 (a)). Our laboratory has also showed that GLP-1 infusion improved regional and global function in patients with myocardial infarction and severe LV systolic dysfunction after successful primary angioplasty (Nikolaidis et al., 2004 (b)). However, neither hemodynamics nor consequent infarct size were altered by GLP-1 infusion in an open chest, anesthetized porcine model of ischemia (Kavianipour et al., 2003). By contrast, GLP-1 infusion reduced infarct size in an isolated isovolumic rodent model of regional ischemia, but ventricular function was unaffected and

glucose uptake was not measured (Bose et al., 2005). As such, whether the actions of GLP-1 on myocardial function and glucose uptake is mediated by direct effects and whether they are evident in both normal and ischemic myocardium remains unresolved.

Accordingly, the purpose of the present study was to determine whether GLP-1 had direct effects on myocardial contractility and glucose uptake in the isovolumic rat heart. A second goal was to examine the cellular effects of GLP-1 on the translocation of glucose transport proteins. A third goal was to compare these effects to those observed following 30 minutes of low flow global ischemia and to determine whether GLP-1 hastened the recovery of ventricular function during reperfusion.

METHODS:

Isovolumetrically heart perfusion

Seventy-five male Wistar-Kyoto rats (Charles River Laboratories) were anesthetized with a 1-ml injection of 60mg/ml pentobarbital sodium. After cessation of peripheral nervous function, hearts were quickly excised and arrested in cold heparin-containing Krebs-Henseleit (KH) buffer. The care and use of animals were conducted under the *Guidelines on Human Use and Care of Laboratory Animals for Biomedical Research* published by the NIH and according to experimental protocol approved by the IACUC Committee of Allegheny General Hospital.

The methodology of Langendorff perfused rat heart preparation has been described previously in details (Zhao et al., 2001). Briefly, hearts were cannulated via ascending aorta for retrograde perfusion at 37 ^{o}C under constant pressure (70 mmHg) using KH buffer containing (in mM) 119 NaCI, 5.4 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 2.5 $CaCl_2$, 25 $NaHCO_3$, 0.25 EDTA and 5.0 glucose. All buffer components were obtained from Sigma (St. Louis, MO). A left atrial incision

was made to expose the mitral annulus through which a water-filled latex balloon was passed into the left ventricle (LV). The balloon was attached via polyethylene tubing to a pressure transducer (Baxter, Model Px 272) that was connected to Triton System I. The initial ballon volume was set to generate left ventricular end diastolic pressure (LVEDP) ≈5 mmHg. Myocardial function was measured including left ventricular developed pressure (LV DevP), LVEDP, and LV dP/dt. LVDevP was calculated by subtracting LVEDP from the peak systolic pressure. Coronary flow was calculated by a timed collection of the effluent.

The perfusate leaving the heart was collected over 1 minute for the measurement of glucose and lactate during steady state every 10-15 minutes during the respective protocols. Glucose and lactate in perfusate was measured using YSI Glucose Analyzer. Glucose uptake was calculated by the following equation (Tada et al., 2000):

Myocardial Glucose Uptake (μmol/min/g) = [G *in*- G *out*] x coronary flow rate (ml / min) / gram dry weight.

G_{in}: glucose concentration, inflow

G_{out}: glucose concentration, outflow

Myocardial lactate production (μmol/min/g) was measured as the product of (Lac_{out} - Lac_{in}) and coronary flow rate (ml/min)/ gram dry weight.

Experimental Protocols

Experimental Protocol 1

After a 30 minute period of stabilization with K-H buffer, hearts were randomly divided into three groups; 1) *Control group (n=15)* - hearts were continuously perfused with K-H for additional 30 minutes; 2) *GLP-1 group (n=15)*: hearts were switched into K-H containing GLP-1

(500 pmol/L) for an additional 30 minutes; 3) *Insulin group (n=15)*: hearts were switched into to K-H containing insulin (100 μU/ml) for an additional 30 minutes. GLP-1 (7-36) amide was synthesized in the protein/peptide core facility of the Endocrine Unit of the Massachusetts General Hospital. The peptide content was 99% pure and gave a single peak on high performance liquid chromatography. The peptide was lyophilized in vials under sterile conditions for single use and was certified to be both pyrogen-free and sterile. Net peptide content was used for all calculations. The peptide used in this protocol was from a single lot. Novolin U-100 insulin was purchased from NovoNordisk (Princeton, NJ)

Experimental Protocol 2

The goal of these experiments was to determine whether GLP-1 mitigates post ischemic contractile dysfunction following brief periods of low flow ischemia. After 30 minutes of perfusion with normal K-H buffer, hearts were subjected to low flow global ischemia (5% of baseline flow by reducing coronary perfusion pressure to 25-30 mmHg) for 30 minutes followed by 30 minutes of reperfusion. The control group (n=10) received KH buffer during low flow ischemia and recovery; The GLP-1 treatment group (n=10) received K-H buffer plus GLP-1 (500 pmol/L), administrated 1 minute prior to inducing low flow ischemia and throughout reperfusion. In order to compare the direct effects of GLP-1 to insulin on post ischemic contractile dysfunction, a third group (n=10) was studied in which insulin (100μU/ml) was added 1 minute prior to the induction of low-flow ischemia and throughout reperfusion. Ventricular function, myocardial glucose uptake, and lactate production were measured every 10-15 minutes during the protocol. Creatine phosphokinase levels were measured on effluent collected at 20 minutes of reperfusion.

Myocardial Insulin Signaling Cascade

LV myocardium from 10 controls, 10 GLP-1 treated and 10 insulin-treated hearts from protocol 1 and 7 controls, 8 GLP-1 treated and 8 insulin treated hearts from protocol 2 were snap frozen in liquid nitrogen and stored at -70°C. Purified sarcolemmal membranes were prepared using density gradient centrifugation as described previously (Nikolaidis et al., 2004 (c)). Translocation of respective GLUT transporters (GLUT-1 and GLUT-4) was analyzed by the assessing the protein content in the purified sarcolemmal membrane preparations as a percent of the total protein expression in the sarcolemma and cytosolic membrane fractions under the respective experimental circumstances.

LV myocardium was homogenized in a buffer free of phosphatase inhibitors and subjected to electrophoresis separation by SDS-PAGE (Nikolaidis et al., 2004 (c)). Resolved proteins were transferred onto PVDF membrane (Immobilon™-P^{SQ}, Millipore Corp., Bedford, MA) at a constant voltage (100V) for 1-2 hours at 4°C. Nonspecific membrane protein binding sites were blocked for at least one hour at room temperature and then membranes were probed with the specific primary antibody overnight at 4ºC. The blots were washed in appropriate secondary antibody-horseradish peroxidase conjugate. The immunoreactive proteins were detected by use of an enhanced horseradish peroxidase/luminol chemiluminescence reaction kit (NEN Life Science Products, Boston, MA) and exposed to X-ray film (Hyperfilm ECL™, Amersham Pharmacia Biotech, Piscataway, NJ). Densitometric analysis of the bands was carried out using a Personal Densitometer SI and ImageQuaNT™ Software (Molecular Dynamics, Sunnyvale, CA). Adjustments for protein loading were accomplished by normalizing bands based upon Comassie staining of the blots.

Goat anti-rabbit IgG-HRP conjugate, anti-Akt-1, anti-phospho-Akt-1 (Ser 473) and (Thr 308) were purchased from BD Transduction Laboratories (San Diego, CA). Anti-GLUT4, anti-

GLUT-1 and Protein A/G-PLUS Agarose were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Phospho-Akt-1 specificity was confirmed using phosphorylated and non-phosphorylated NIH/3T3 cell extracts purchased from Cell Signaling Technology, Inc. (Beverly, MA). Anti-GLP-1 receptor antibody was purchased from Alpha Diagnostics International (San Antonio, Tx).

The AMPK activity in the LV myocardium was determined using the method of Musi et al (2001). In brief, the LV lysates containing 200µg protein were immunoprecipitated with a specific antibody to AMPKα2 and Protein A/G PLUS-Agarose beads (Santa Cruz Biotechnology, Santa Cruz, CA). Kinase reactions were performed in 40 mM HEPES, pH 7.0, 0.2 mM AMP, 80 mM NaCl, 0.8 mM DTT, 5 mM $MgCl_2$, 0.2 mM ATP (containing 2 µCi [^{32}P]γ-ATP), and 0.2 mM SAMS peptide in a final volume of 40 µl for 20 min at 30°C. At the end of the reaction, a 20-µl aliquot was removed and spotted on Whatman P81 paper. Radioactivity was quantitated with a scintillation counter. Activity was expressed as incorporated ATP (picomoles) per milligram of protein per minute.

The p38α MAPK activity in the LV myocardium was determined using a p38α/SAPK2a Assay Kit purchased from Upstate (Charlottesville, VA) with slight modifications. In brief, the LV lysates containing 200µg protein were immunoprecipitated with a specific antibody to p38α MAPK (Chemicon, Temecula, CA) and Protein A/G PLUS-Agarose beads (Santa Cruz Biotechnology, Santa Cruz, CA). The activation of MAPKAP Kinase 2 was carried out by adding 5 µl (200ng) unactive MAPKAP Kinase 2 and then 5 µl ice cold 200 µM ATP/30mM $MgCl_2$ in ADB1, and incubating for 15 minutes at 30°C. The activation reaction was stopped by adding 40 µl ice-cold ADB1 and 10 µl 0.086 mM MAPKAP 2 Kinase substrate peptide. The phosphorylation of MAPKAP Kinase 2 substrate peptide was performed by adding 10 µl of 75

mM $MgCl_2$ and 500 µM ATP (containing 5 µCi [^{32}P]γ-ATP) in a final volume of 80 µl for 10 min at 30°C. At the end of the reaction, a 20-µl aliquot was removed and spotted on Whatman P81 paper. The papers were washed three times in 1% phosphoric acid and one time with acetone. Radioactivity was quantitated with a scintillation counter. Activity was expressed as incorporated ATP (picomoles) per milligram of protein per minute.

Total nitric oxide production in the isolated preparation was determined by measuring NOx in the effluent following control, GLP-1, and insulin infusions, respectively. The measurements of total nitric oxide (NO) levels were carried out using a kit purchased from R&D Systems, Inc. (Minneapolis, MN). Reaction buffer was ultrafiltered through a 10,000 molecular weight cutoff filter to eliminate proteins. Since most of the NO is oxidized to nitrite (NO_2^-) and nitrate (NO_3^-), the concentrations of these anions are used as a quantitative measurement of NO production. After the enzymatic conversion of nitrate to nitrite by nitrate reductase, the spectrophotometric measurement at 540 nm of NO_2^- is accomplished by using the Greiss Reaction. Standard curves of known nitrite levels were used and total NO values of experimental samples were determined using a linear curve-fit analysis.

Statistics

All measurements are expressed as means ± SEM. The data were analyzed by either unpaired Student's t-test or one-way ANOVA. The Student-Newman-Keuls post hoc test was further used for pairwise comparisons after ANOVA demonstrated a significant difference between groups. Paired t-test was used to compare any pair of pre- and post treatment values for the same parameter. $p < 0.05$ was considered significant.

RESULTS:

The Effects of GLP-1 on left ventricular function in normal hearts:

The baseline functional and metabolic parameters were comparable between groups **(Table 1**). In the Control group, a continuous perfusion with KH buffer did not alter baseline LV DevP, LV dP/dt, heart rate and coronary flow (**Figure 1**). When hearts were switched at 30 minutes from normal K-H buffer to the buffer containing GLP-1 (500 pmol/L), GLP-1 caused a rapid decrease in left ventricular developed pressure (Baseline: 100 ± 2 mmHg; GLP-1: 75 ± 3 mmHg, $p<0.05$), associated with a decline in LV dP/dt max (Baseline: 4,876±65 mmHg/sec; GLP-1: 4,353±76 mmHg/sec, $p<0.05$, **Figure 1**). GLP-1 increased coronary flow (Baseline: 14±1 ml/min; GLP-1: 18±2 ml/min, $p<0.05$)**,** but did not change left ventricular diastolic pressure or heart rate.

To compare the effects to GLP-1 to insulin, we studied a third group using a similar protocol, but added insulin (100 μU/ml) instead of GLP-1 at 30 minutes. There was a significant increase in LV DevP (Baseline: 98 ± 3 mmHg; Insulin: 108± 3 mmHg, $p<0.05$) and LV dP/dt_{max} (Baseline: 4981±98 mmHg/sec; Insulin: 5101 ±78 mmHg/sec, $p<0.05$) in contrast to the findings with GLP-1 (**Figure 1**). Additionally, insulin administration increased coronary flow (Baseline: 14±3; Insulin: 19±2 ml/min, $p<0.05$)**,** but did not change LVEDP or heart rate (**Figure 1**).

Both GLP-1 and insulin administration increased myocardial glucose uptake compared to Control (**Figure 2**). The increase in glucose uptake was associated with increased glucose extraction and was greater than the observed increase in coronary flow. The increase in myocardial glucose uptake was accompanied by increased myocardial lactate production

compared to Control, although the magnitude of lactate production was less and occurred later than the increase in glucose uptake.

Effects of GLP-1 on Glucose transporters

Figure 3 illustrates the effects of GLP-1 on selective components of the insulin-signaling cascade. Importantly, the 43 KDa isoform of the GLP-1 receptor was present in the myocardium, and did not differ significantly between Control (30±8 DU), GLP-1 (42±10 DU) and insulin (50±12 DU) treated groups. Despite the observed increase in myocardial glucose uptake, GLP-1 treatment was not associated with either increased Akt-1 abundance or activation (phospho-Akt-1) compared to Control, while insulin treatment was associated with a marked increase in the serine 473 and threonine 308 phosphorylation of Akt-1. As expected, insulin stimulated GLUT-4 translocation to a greater extent than either Control or GLP-1 treated groups. In contrast, GLP-1 significantly increased myocardial NOx production, p38 MAP kinase activity and GLUT-1 translocation to a greater extent than in the Control or insulin treated groups (**Figure 3**).

The Effect of GLP-1 on left ventricular function during low flow ischemia.

GLP-1 significantly improved the recovery of function following 30 minutes of low flow ischemia (**Figure 4**). LV DevP (Control: 66±6; GLP-1: 84±5, $p<0.05$) and LV dP/dt max (Control: 2,345 ± 112 mmHg/sec; GLP-1: 4,081 ±165 mmHg/sec, $p<0.05$) recovered faster and to a greater extent than Control. GLP-1 treatment was associated with a lower post-ischemic LVEDP (Control: 13±4 mmHg; GLP-1: 4±2 mmHg, $p<0.05$). Heart rate and coronary flow responses did not change significantly with GLP-1 administration. The effects of GLP-1 did not

differ significantly from the effects of insulin on the recovery of LV function following ischemia.

GLP-1 was associated with an increase in post-ischemic myocardial glucose uptake compared to Control and significantly greater increase in ischemic lactate production (**Figure 5**). The effects of GLP-1 were similar to the effects of insulin.

Given that the ischemic insult involved global as opposed to regional myocardial perfusion, we measured creatine phosphokinase (CPK) release following reperfusion. The CPK release was less ($p<0.05$) in the GLP-1 treated group (187±43 U/L) and the insulin treated group (197±35 U/L) compared to control (358±31 U/L).

The Effects of GLP-1 on Post Ischemic GLUT Translocation

Figure 6 illustrates the effects of GLP-1 on GLUT-1 and GLUT-4 translocation following 30 minutes of reperfusion compared insulin or Control. There was no difference AMP kinase activity among the groups (Control: 26±5; GLP-1: 28±7; Insulin: 31±10 pmol ATP/mg/min), although activity was higher ($p<0.05$) in the post ischemic myocardium compared to normal hearts (Control: 13±5; GLP-1: 17±8; Insulin: 15±7 pmol ATP/mg/min). Notably, both the GLP-1 and insulin treated groups demonstrated increased GLUT-4 and GLUT-1 translocation compared to Control, but in the absence of increased Akt-1 expression or activation. However, both GLP-1 and insulin treated groups showed increased myocardial NO production and increased p38 MAP kinase activity in contrast to the selective effects on glucose transporters observed in normal hearts.

Discussion:

In the present study, we determined that GLP-1 had direct negative effects on myocardial contractility, but stimulated myocardial glucose uptake and lactate production in normally

perfused, isolated rat hearts. The effects on myocardial contractility were distinct from those of insulin, despite comparable effects on coronary flow and myocardial glucose uptake. We identified the GLP-1 receptor in the myocardium and demonstrated that the effects of GLP-1 to stimulate myocardial glucose uptake were not mediated through the classical insulin-signaling cascade involving Akt-1 activation and GLUT-4 translocation, but through increased myocardial nitric oxide production, p38 MAP kinase activity and GLUT-1 translocation. Finally, GLP-1 enhanced functional recovery of the post-ischemic myocardium in contrast to its negative inotropic effects on the normally perfused heart. Both the functional and the cellular effects on glucose uptake and lactate production were similar to those of insulin in the post-ischemic heart.

There have been a limited number of studies examining the effects of GLP-1 on infarct size or recovery from a post ischemic injury with conflicting results. Kavianpour et al., (2003) reported that GLP-1 (7-36) did not alter myocardial glucose utilization, hemodynamics, or infarct size in an acute porcine model of infarction, despite the fact that GLP-1 increased insulin secretion and decreased lactate uptake. In contrast, our laboratory (Nikolaidis et al., 2005) has demonstrated that a twenty-four hour infusion of GLP-1 (2.5 pmol/kg/min) mitigated post ischemic contractile dysfunction and improved ventricular relaxation in conscious dogs following brief periods (10 minutes) of complete coronary artery occlusion. Similar beneficial effects have been observed in humans treated with a seventy-two hour infusion of a similar dose of GLP-1 following acute myocardial infraction complicated by cardiogenic shock (Nikolaidis et al., 2004 (b)). Nonetheless, these studies do not address whether the effects of GLP-1 were attributable to direct myocardial actions or indirect effects associated with the systemic insulinotropic actions of GLP-1.

Bose et al (2005) investigated the effects of GLP-1 infusion (4.8 pmol/kg/min) *in vitro* in Sprague Dawley rats subjected to 30 minutes of LAD occlusion and 120 minutes of reperfusion and observed that GLP-1 had no salutary effects on hemodynamics. The absence of hemodynamic benefits was surprising given that the investigators found a 50% reduction in infarct size in the GLP-1 treated group. A major difference between our findings and those of Bose et al. (2005) in the isolated heart models during ischemia may relate to the difference between complete and partial ischemia in the two models. The low flow global ischemia employed in our model may have facilitated GLP-1 perfusion into the ischemic zone whereas Bose et al. (2005) used a model of complete coronary artery occlusion. While the results differ, both studies support a direct effect of GLP-1 on myocardial function and preservation.

We observed abundant expression of GLP-1 receptors in the rat myocardium. However, the mechanisms whereby GLP-1 receptors couple to intracellular effectors in extra-pancreatic tissues, such as the heart, remain largely unexplored. We observed opposing effects of GLP-1 and insulin on myocardial contractility in normal hearts. In turn, we observed that GLP-1 and insulin had comparable effects on myocardial glucose uptake, but via different cellular mechanisms. Insulin mediated glucose uptake was associated with Akt-1 phosphorylation and GLUT-4 translocation. In contrast, GLP-1 did not increase phospho-Akt-1 or GLUT-4 translocation, but did result in increased GLUT-1 expression in the sarcolemma. Furthermore, GLP-1 was associated with increased myocardial NO production and increased p38 MAP kinase activity. Prior studies have demonstrated that GLP-1 increases NO production in vascular smooth muscle (Nystrom et al., 2004; Nystrom et al., 2005). However, this is the first illustration of such an effect in myocardium. Importantly, NO has been shown to stimulate p38 MAP kinase (McFalls, 2004) and both NO (Van Dyke, 2003) and p38 MAP kinase (Denton, 1995; Taha,

1997) has been shown to increase membrane associated GLUT-1 in isolated muscle cells. It remains to be determined what the proximal steps are in GLP-1 receptor coupling in the myocardium and where the contractile and glucoregulatory pathways diverge.

During recovery from low flow ischemia, we showed that both GLP-1 and insulin had comparable effects on myocardial glucose uptake and functional recovery from the ischemic insult. Notably, both GLP-1 and insulin mediated myocardial glucose uptake did not involve Akt-1 activation in the post ischemic myocardium. Our findings on the effects of GLP-1 on Akt-1 activation differ from those reported by Bose (2005). Prior studies (Young, 1997; Erert, 1999) have shown that low flow ischemia is associated with increased myocardial GLUT-1 and GLUT-4 expression. Notably, during recovery, all three groups had increased AMP kinase activity consistent with ATP depletion during low flow ischemia. While AMP kinase is a potent stimulus to insulin-independent GLUT-4 translocation, this mechanism is insufficient to explain the increases in membrane GLUT-1 expression in the GLP-1 and insulin treated groups. However, both GLP-1 and insulin treated groups demonstrated increased NO production and p38 MAP kinase activity in the post-ischemic hearts compared to Control and in contrast to what was observed with insulin in the normal heart. This pathway has been demonstrated previously to be integral to post ischemic functional recovery (McFalls, 2004). It remains to be elucidated as to how GLP-1 increases nitric oxide production, the isoforms involved, and the intracellular compartment in which nitric oxide synthase is activated. Finally, it is conceivable that the benefits of GLP-1 and insulin to improve post-ischemic contractile dysfunction are related simply to enhanced glucose uptake rather than a direct cellular effect on myocardial stunning. Our findings provide new insights into the direct myocardial effects of GLP-1 and novel signaling mechanisms for glucose uptake, independent of the Akt-1 activation.

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Legends for Figures:

Figure 1: The effects of GLP-1 or insulin on Left ventricular developed pressure (LVDevP), LV dP/dt, heart rate and coronary flow compared to KH buffer alone (Control, n=15). GLP-1 (500pmol/L, n=15) or Insulin (100μU/ml, n=15) was added at 30 minutes (arrow) at constant perfusion pressure. * $p<0.05$ GLP-1 or Insulin infusion compared to Control.

Figure 2: The effects of GLP-1 or insulin on myocardial glucose uptake and myocardial lactate production in isolated rats compared to KH buffer alone (Control, n=15). GLP-1 (500pmol/L, n=15) or Insulin (100μU/ml, n=15) was added at 30 minutes (arrow) at constant perfusion pressure. * $p<0.05$ GLP-1 or Insulin infusion compared to Control.

Figure 3: The myocardial signal transduction associated with increased myocardial glucose uptake in response to GLP-1 (n=10) or Insulin (n=10) compared to buffer alone (Control, n=10).

Figure 4: The effects of GLP-1 or Insulin on coronary flow, LV developed pressure (LVDevP), LVdP/t, and LV end diastolic pressure (LVEDP) during 30 minutes flow ischemia (5% of baseline), and 30 minutes of reperfusion compared to KH buffer alone (Control, n=10). GLP-1 (500 pmol/L, n=10) or Insulin (100 μU/ml, n=10) was added to the perfusate 1 minute prior to low flow ischemia and continued through reperfusion. * $p<0.05$ GLP-1 or Insulin compared to Control.

Figure 5 The effects of GLP-1 or Insulin on myocardial glucose uptake and myocardial lactate production during 30 minutes flow ischemia (5% of baseline), and 30 minutes of reperfusion (Control, n=10). GLP-1 (500 pmol/L, n=10) or Insulin (100 μU/ml, n=10) was added to the perfusate 1 minute prior to low flow ischemia and continued through reperfusion. * $p<0.05$ GLP-1 or Insulin compared to Control.

Figure 6: The myocardial signal transduction associated with increased myocardial glucose uptake in the post ischemic myocardium. * $p<0.05$ GLP-1 (n=8) or Insulin (n=8) compared to Control (n=7).

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Table 1 Baseline Hemodynamics

	Control (*n=10*)	**GLP-1 (*n=10*)**	**Insulin (*n=10*)**
LV Systolic Pressure (mmHg)	101 ± 2	104 ± 3	105 ± 4
LV End Diastolic Pressure (mmHg)	5 ± 1	4 ± 1	5 ± 1
LV dp/dt (mmHg/sec)	4,978 ± 76	4,876 ± 65	4,981 ± 98
LV Dev P (mmHg)	95 ± 2	100 ± 3	98 ± 3
Heart Rate (min^{-1})	251 ± 16	247 ± 13	236 ± 18
Coronary Flow (ml/min)	13 ± 1	14 ± 2	14 ± 3
Myocardial Glucose Uptake (μmol/min/g)	35 ± 4	33 ± 3	32 ± 4
Myocardial Lactate Production (μmol/min/g)	1.8 ± 0.1	1.7 ± 0.2	1.7 ± 0.2

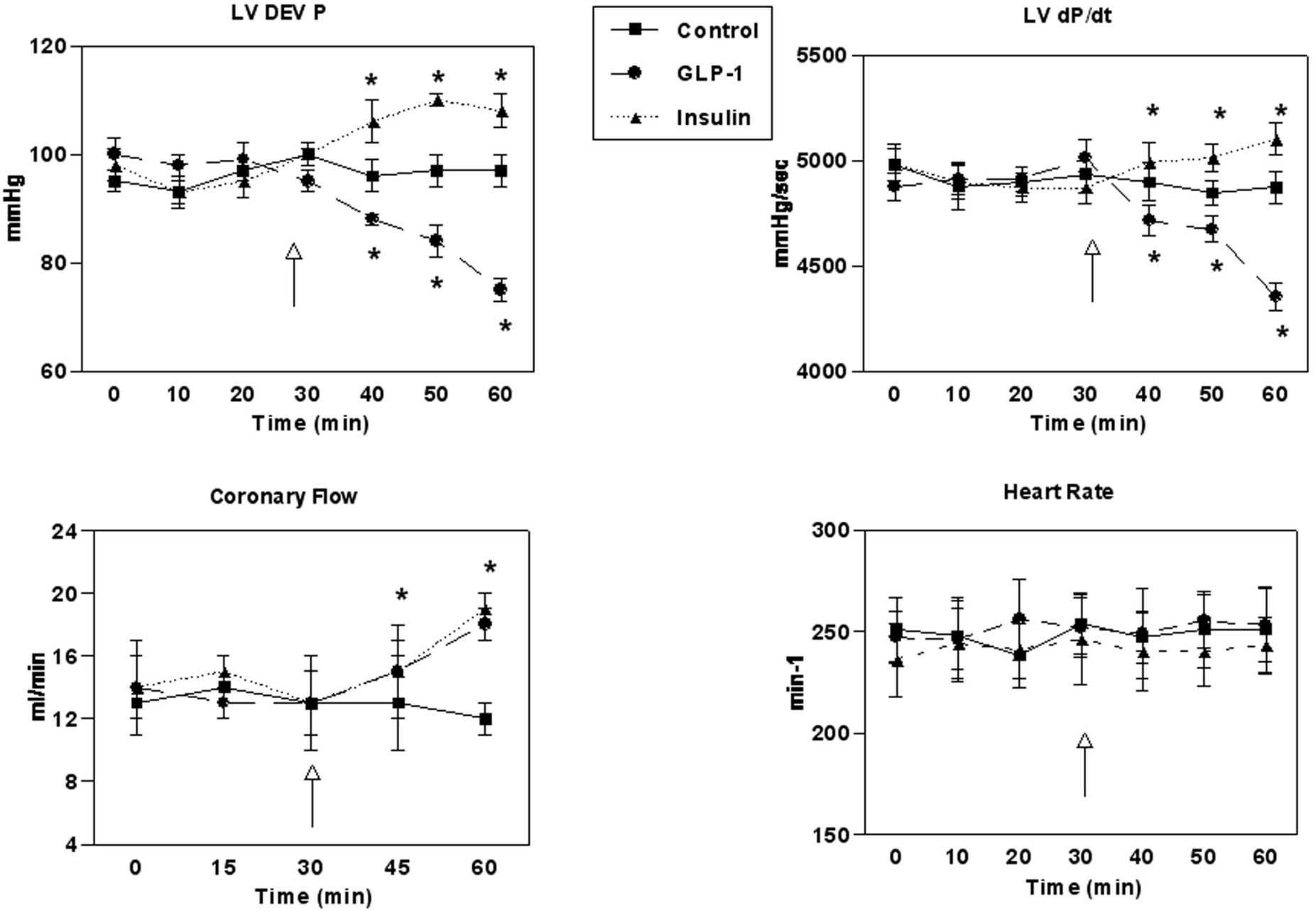
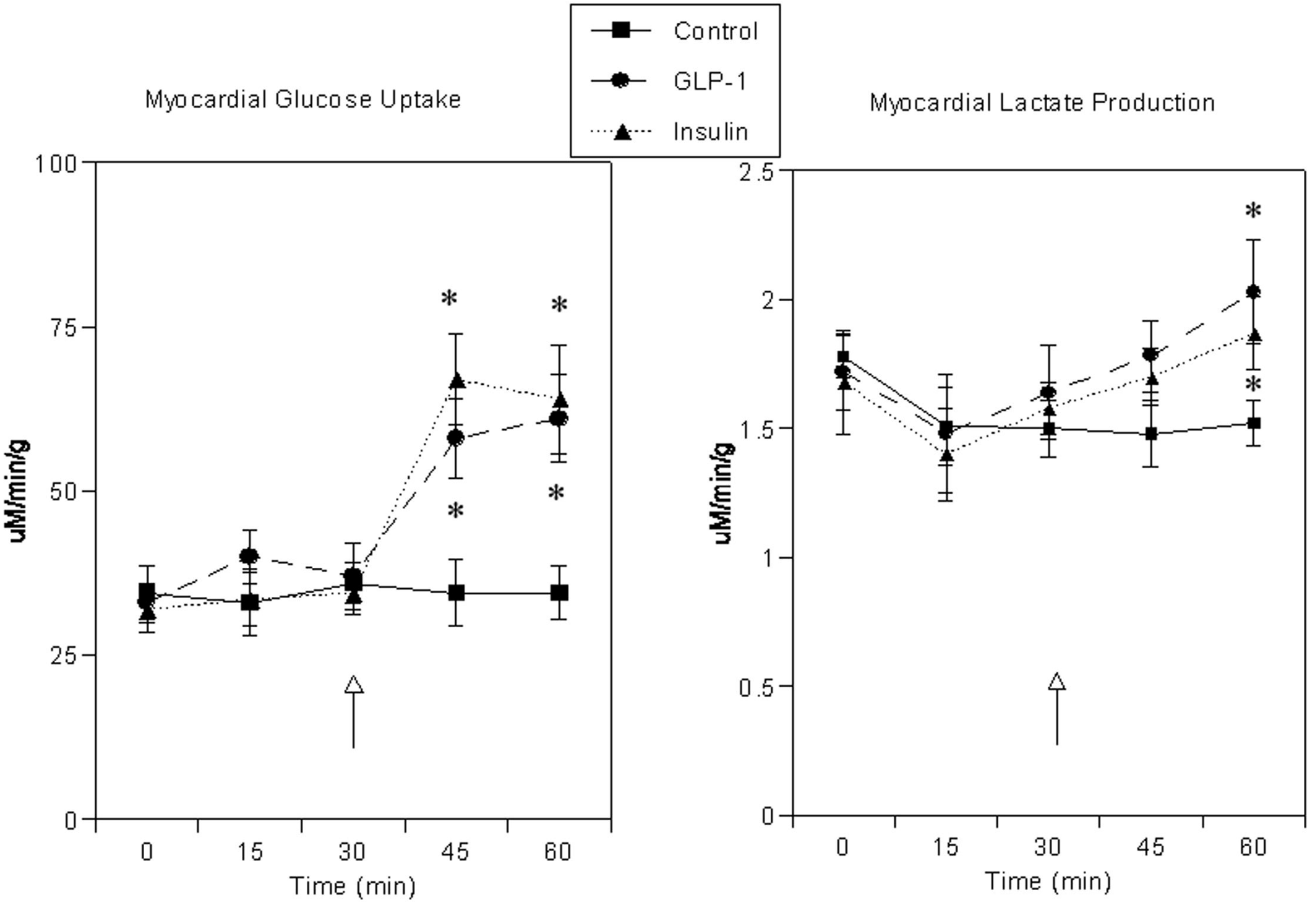


Figure 1



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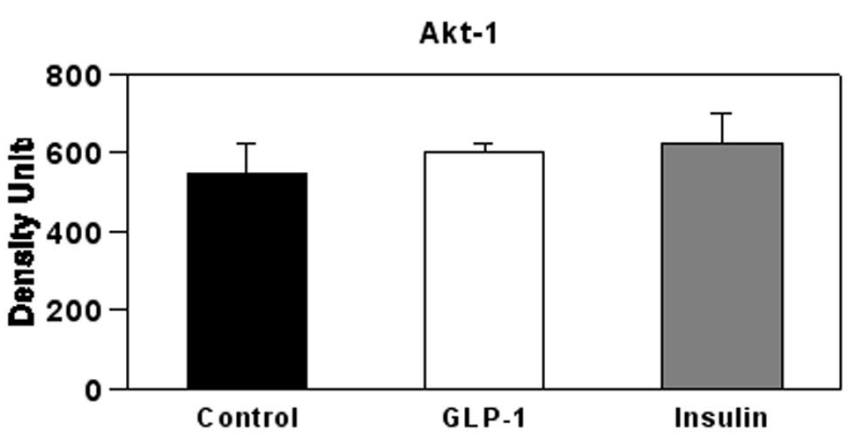
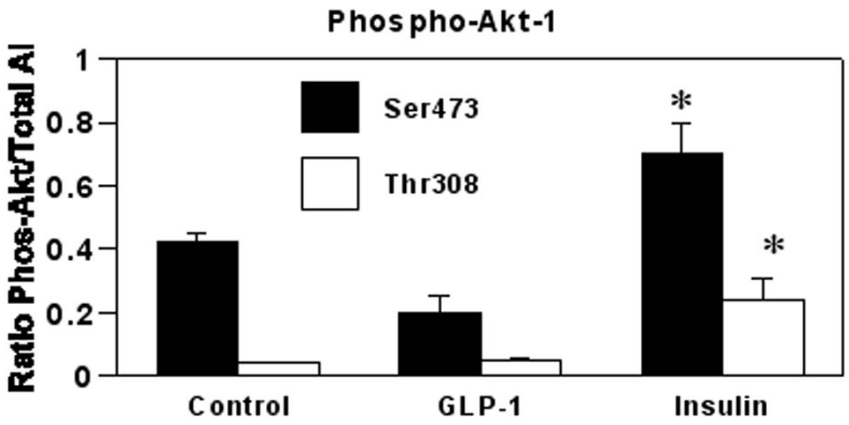
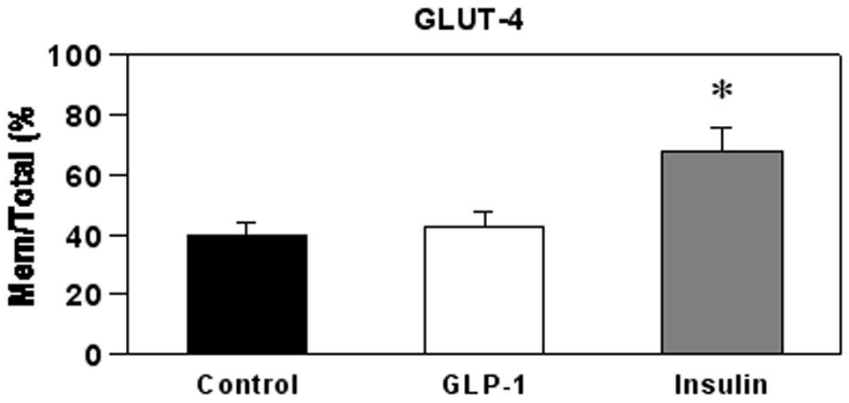
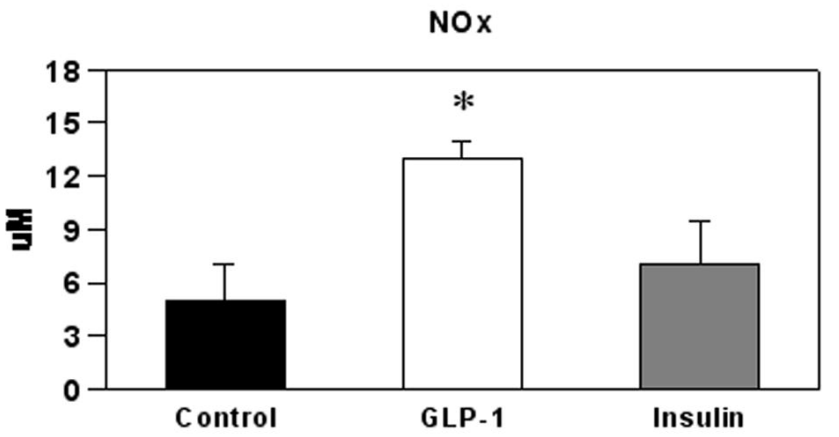
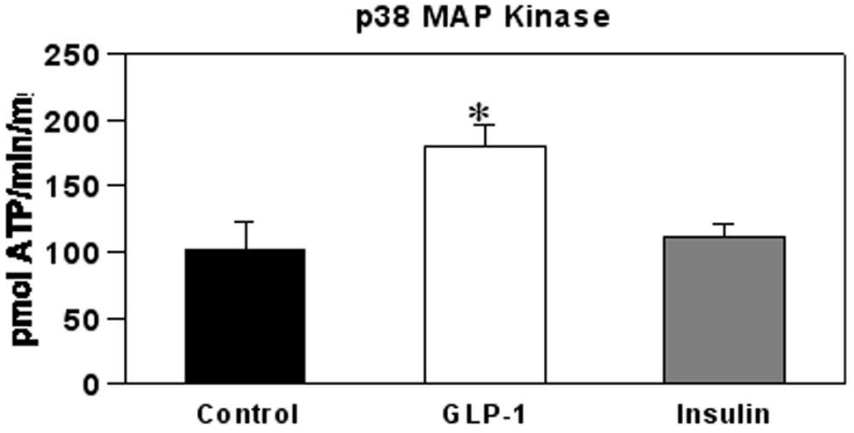










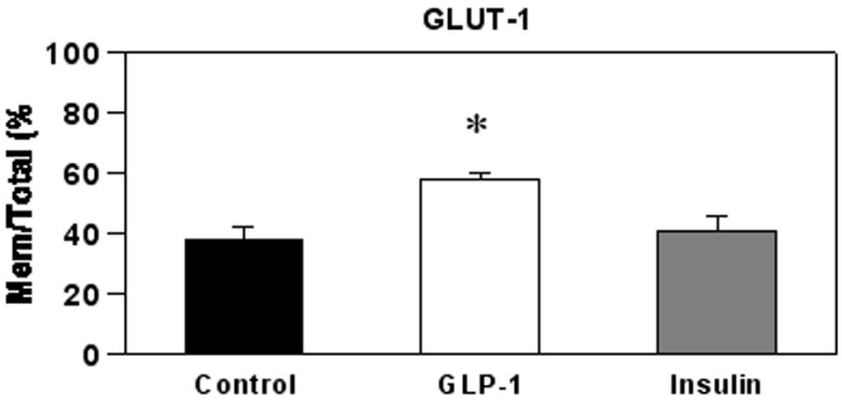
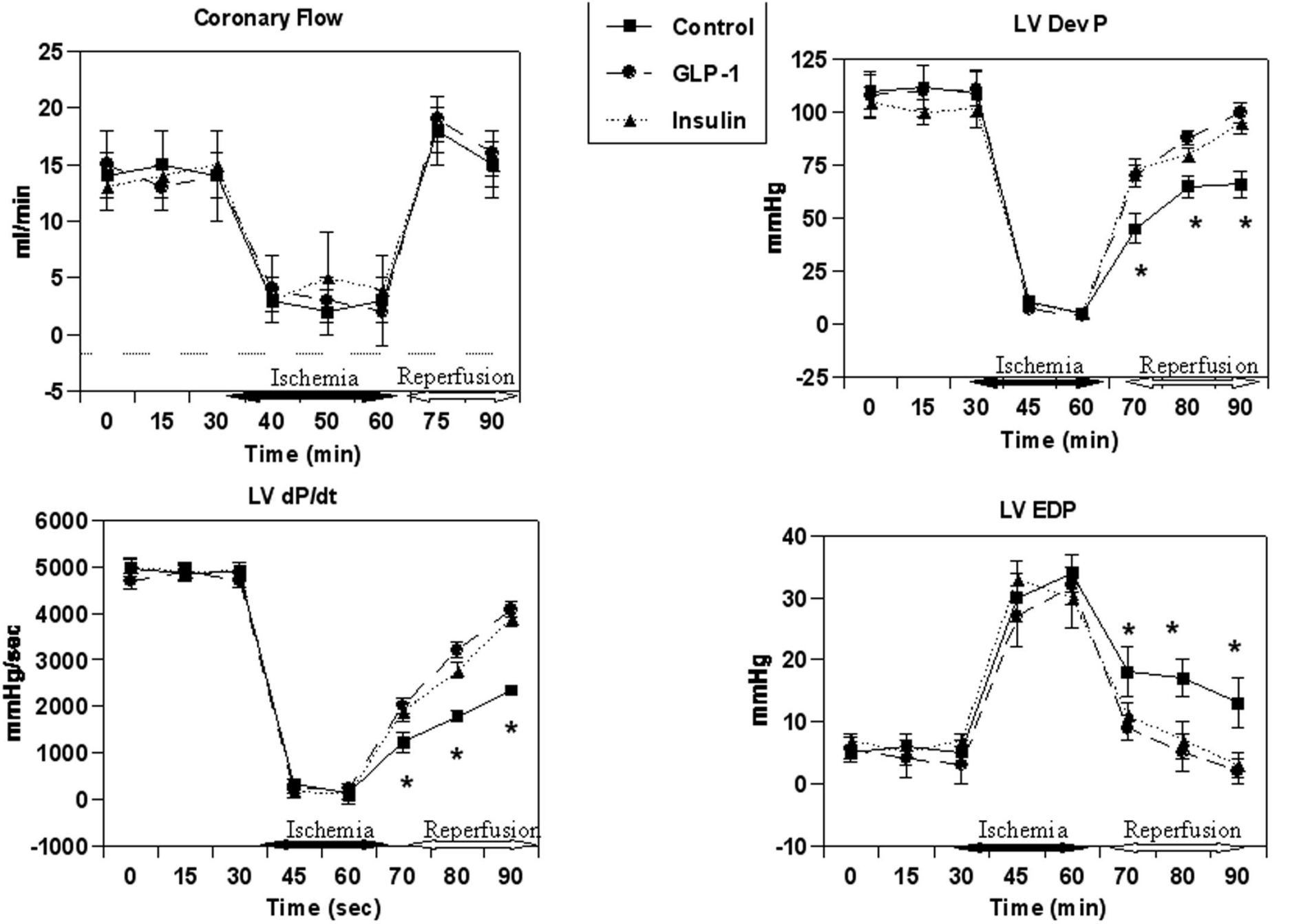


Figure 3



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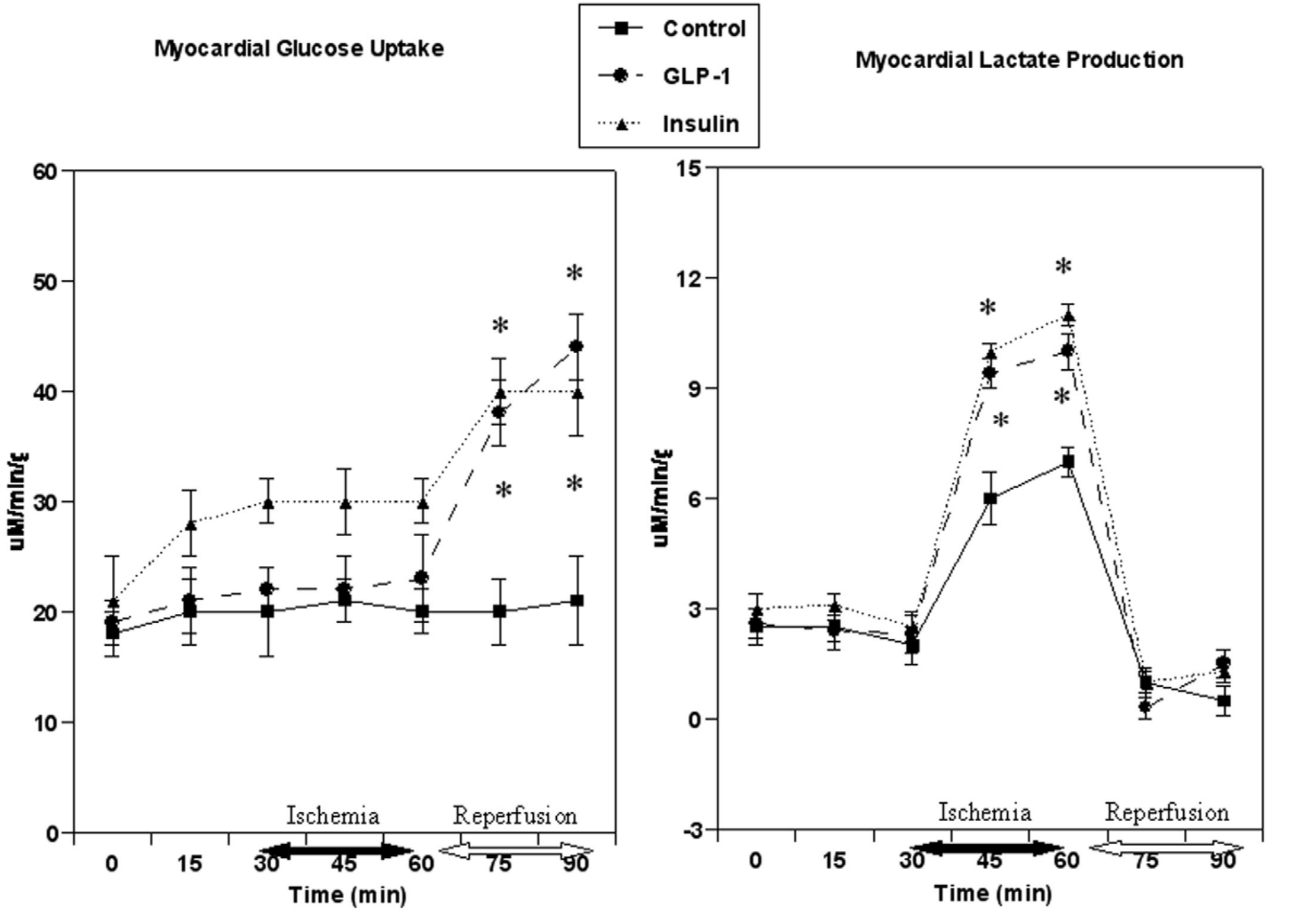

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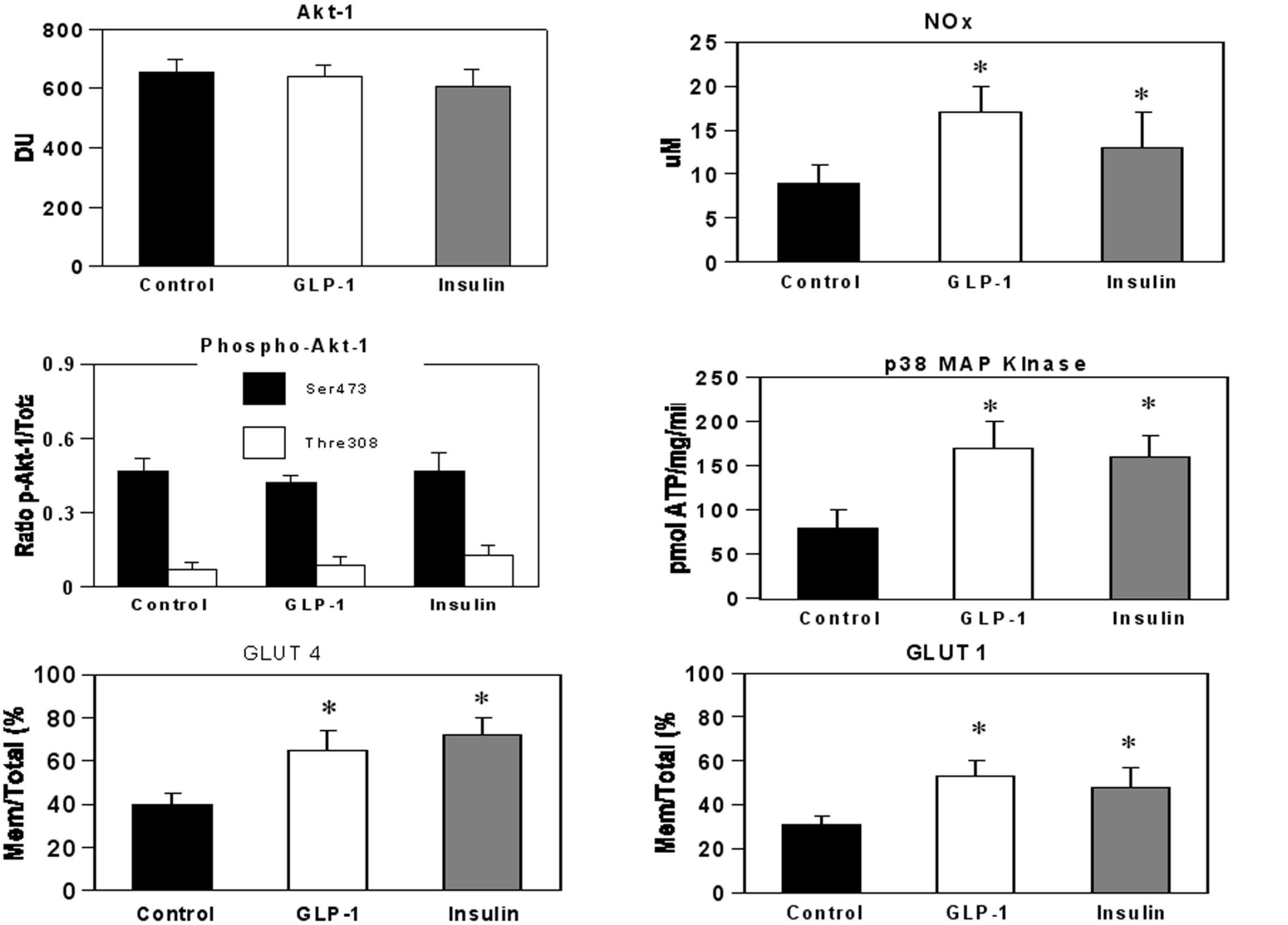

Figure 6