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Chronic Glucagon-like Peptide-1 (GLP-1) Infusion Sustains LV Systolic Function and Prolongs Survival in the Spontaneously Hypertensive Heart Failure Prone Rat

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Abstract

Background—GLP-1 treatment leads to short term improvements in myocardial function in ischemic and non-ischemic cardiomyopathy. It is unknown whether GLP-1 improves survival when administered over a longer time period. Spontaneously hypertensive, heart failure prone rats (SHHF) progress to advanced heart failure and death over a 15 month period. We sought to determine whether a continuous infusion of GLP-1 would reduce mortality in this model.

Methods and Results—At 9 months of age, 50 SHHF rats were randomized to receive a 3-month continuous infusion of either GLP-1 or saline. Metabolic parameters were measured and cardiac ultrasounds performed at study initiation and completion of treatment. Surviving rats were euthanized at 12 months. Hearts were perfused in an isolated, isovolumic heart preparation and Tunel staining of myocardial samples was performed. Baseline metabolic and cardiac functional parameters were comparable. GLP-1 treated SHHF had greater survival (72% vs. 44%, $p=0.008$) at 12 months of age. In addition, GLP-1 treatment led to higher plasma insulin, lower plasma triglycerides, and preserved LV function. GLP-1 treated rats demonstrated decreased myocyte apoptosis by Tunel staining, as well as reduced caspase-3 activation. No increase in p-BAD expression was seen. In isolated hearts, the LV systolic pressure and developed pressure were greater in the GLP-1 group. Myocardial glucose uptake was also increased in GLP-1 treated SHHF.

Conclusions—Chronic GLP-1 treatment prolongs survival in obese, hypertensive SHHF. This is associated with preserved LV function and LV mass index, increased myocardial glucose uptake and reduced myocyte apoptosis.

Keywords

apoptosis; glucose; heart failure; mortality

Introduction

Glucagon like peptide-1 (GLP-1 7-36 amide) is a member of the pro-glucagon family of incretin peptides, implicated in the control of appetite and satiety. These agents have been shown to have significant clinical efficacy in the treatment of type 2 diabetes mellitus^{1,2,3}. Prior studies have demonstrated that GLP-1 infusion has salutary cardiovascular effects in the presence or

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absence of diabetes, suggesting that GLP-1 may have cardioprotective effects independent of those attributable to tight glycemic control⁴. Acute infusions of GLP-1 were associated with enhanced recovery following brief ischemic insults in conscious dogs⁵ and in intact⁶ and isolated rat hearts^{6,7}. Seventy-two hour infusions of GLP-1 have been associated with increased myocardial glucose uptake and improved left ventricular (LV) function in conscious dogs with dilated cardiomyopathy induced by rapid pacing⁸. Similar benefits have been seen in patients following acute MI⁹. However, to date these studies have involved relatively brief infusions (days) and consequently have assessed only short-term improvements in cardiac performance in post ischemic or cardiomyopathic states. There have been no longer-term studies to determine whether these acute cardiovascular benefits of GLP-1 are sustainable. Moreover, there are no studies to determine whether chronic GLP-1 infusion improves survival in experimental or human models predisposed to cardiovascular mortality.

The spontaneously hypertensive, heart failure prone rat is a model of obesity, insulin resistance, and hypertension that progresses to a dilated cardiomyopathy over a 12–15 month period¹⁰. The progressive development of the cardiac phenotype is associated with the onset of glucose intolerance. Treatment with both standard and novel heart failure therapies has been shown to moderate the development of LV dysfunction^{11,12,13,14}. As such, the model constitutes an attractive opportunity to study the long-term effects of GLP-1 on myocardial glucose uptake, cardiac structure and function, and survival. Accordingly, the purpose of the present study was to determine the effects of a chronic (3 month), continuous infusion of GLP-1 (7-36) amide on metabolic parameters in mature SHHF rats. A second goal was to determine the effects of chronic GLP-1 infusion on LV systolic function and myocardial structure. Last, we sought to determine whether and by what mechanism GLP-1 may increase survival in this model vulnerable to premature cardiac mortality from progressive LV systolic dysfunction.

Methods

Seventy SHHF were purchased from Charles River Laboratories at 6 months of age and acclimated to the laboratory on a standard rat chow limited to 15 pellets per day to standardize caloric intake for three months prior to study. At 9 months of age, the rats were divided randomly into two cohorts; control and GLP-1. Ten rats in each cohort underwent baseline studies to define the metabolic, morphometric and hemodynamic parameters at the time point. The remaining rats were randomized to receive either GLP-1 (1.5 pmol/kg/min, n=25) or an equivolume of saline as control (n=25) administered by intraperitoneal infusion using a catheter attached to an Alzet mini-pump inserted into the subcutaneous fat. Pumps were replenished every 21 days. The rats were followed for 3 months and those that survived underwent similar analysis at 12 months. 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 National Institutes of Health and according to experimental protocol approved by the Institutional Animal Care Use Committee of Allegheny General Hospital.

Metabolic Parameters

Fasting plasma insulin, glucose, NEFA, adiponectin, leptin and triglyceride levels were obtained at the time of euthanasia at either 9 (baseline) or 12 months. Blood was drawn in ice-cold tubes containing EDTA as an anticoagulant and Trasylol (Bayer Corp., West Haven, CT) and Diprotin A (Bachem Bioscience, King of Prussia, PA) as protease inhibitors. The samples were kept on ice and centrifuged within 30 minutes of collection at 1000×g for 10 minutes. The plasma was removed and stored at -80°C. The metabolic parameters were assayed as described previously⁸. Plasma leptin and adiponectin were assayed using the Linco leptin assay kit (Linco Research immunoassay, St. Charles, MO) on plasma stored at -20°C. The plasma concentration of total GLP-1 (including both the (7-36) and (9-36) amides) was determined in

plasma collected in ice-cooled tubes containing EDTA and a DPP-IV inhibitor using an antibody directed against the COOH-terminal portion of the peptides (Linco Research, St. Charles, MO).

Echocardiography

M-mode and 2-D echocardiography was performed under light isoflurane anesthesia in 10 rats in each group at baseline (9 months) and in the surviving animals at 12 months. Measurements of left ventricular (LV) dimensions in end-diastole (LVEDD) and end-systole (LVESD), as well as respective measurements of the inter-ventricular septum (IVS) and posterior wall (PW) in systole and diastole were recorded. The modified Quinones¹⁵ and Deveraux¹⁶ equations were utilized to calculate left ventricular ejection fraction (LVEF) and left ventricular mass (LVM) respectively. The volumetric and LV indices were normalized to body mass to account for the effect of obesity in these rats. The echo reader was blinded to the treatment groups.

Isolated Isovolumic Heart Preparations

The Langendorff methodology of isolated perfused rat hearts has been described previously in detail⁷. Briefly, hearts were cannulated via ascending aorta for retrograde perfusion at 37 °C under constant pressure (74 mmHg) using KH buffer containing (in mM) 119 NaCl, 5.4 KCl, 1.2 MgSO₄, 1.2 KH₂PO₄, 2.5 CaCl₂, 25 NaHCO₃, 0.25 EDTA, 5.0 mM glucose and 40 μU/ml of insulin. 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 balloon volume was set to generate left ventricular end diastolic pressure (LVEDP) ≈ 5 mmHg. Myocardial function was measured including left ventricular developed pressure (LV Dev P), LVEDP, and LV dP/dt. LV Dev P was calculated by subtracting LVEDP from the peak systolic pressure. Coronary flow was calculated by a timed collection of the effluent.

For the measurement of myocardial glucose uptake during steady state, the perfusate leaving the heart was collected over 1 minute every 10-15 minutes. Glucose concentration in the effluent was measured using YSI Glucose Analyzer. Glucose uptake was calculated as described previously^{7,17}.

Myocyte Apoptosis

TUNEL staining was performed on sections of LV myocardium to quantify myocyte and non-myocyte apoptosis. DAPI staining was used to identify nuclei as a control. The percent of apoptotic cells was calculated using the Metamorph system. Western blot analysis of whole heart homogenate was performed as described previously¹⁸ to determine protein levels of the activated form of caspase -3 and phosphorylated form of BAD at serine¹³⁶. The anti-caspase 3, anti-BAD and anti p-BAD serine¹³⁶ antibodies were obtained from Cell Signaling Technology and Santa Cruz Biotechnology, respectively.

Insulin Signaling

At baseline (9 months) and in surviving rats at 12 months, subsets of rats were euthanized, the myocardium was removed and LV samples were snap frozen in liquid nitrogen. Components of the myocardial insulin signaling cascade were assayed by Western blot using rat-specific antibodies in purified sarcolemmal membrane preparations generated by density gradient centrifugation, as previously described from our laboratory¹⁸. In half of the rats within each group, insulin (100 U SQ) was administered 5 minutes prior to removing the heart, in order to assess the functional response of insulin signaling proteins under physiological stimulation. The other half of the rats in each group were used to investigate the integrity of these cellular

pathways under basal metabolic conditions. Immunoblotting for IR- β , IRS-1, Akt-1 total protein expression, serine (Ser 472, 473) and threonine (Thr 308) phosphorylated Akt-1, and serine (307) phosphorylated IRS-1 was conducted using specific antibodies as described previously¹⁸. All Western blots were performed using 10 μ g protein/lane. Adjustment for protein loading was accomplished by normalizing bands based on Coomassie staining of the blot.

For the measurement of GLUT-4 translocation, purified light (sarcolemmal) and heavy (intracellular) membrane vesicles were isolated from LV myocardium using a sucrose gradient^{7,18}. Membranes were probed with rabbit anti-GLUT4 polyclonal antibody (1:1000) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) overnight at 4°C. Densitometric analysis of the bands was carried out using a Personal Densitometer SI and ImageQuaNT™ Software (Molecular Dynamics, Sunnyvale, CA) and expressed as the % sarcolemmal membrane to total GLUT-4¹⁸. The signaling data were expressed as the % insulin stimulated in each group at the two time points.

Statistical analysis

All measurements are expressed as means \pm SEM. The data were analyzed by unpaired Student's t-test with a Bonferroni correction applied for multiple comparisons. The survival response with respect to time between groups was analyzed by two-way ANOVA. P values were provided for all comparisons. Statistical significance was indicated when $p < 0.05$.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

LV weight, body weight and metabolic parameters

There was no difference in body weight, body mass index, LV mass or the LV mass index between the groups at baseline, nor were there differences in baseline metabolic parameters, with both groups demonstrating significant fasting hyperglycemia at 9 months of age (Table 1). GLP-1 treatment was associated with a significant reduction in body weight and body mass index and a trend ($p=0.061$) toward an increase in LV mass index over the three month period of treatment. These responses were significantly different compared to the response over time in the saline control group. There were significant declines in plasma glucose and triglyceride levels, as well as significant increases in plasma insulin and adiponectin levels in the GLP-1 group during the three month period of treatment. These responses as well as the glucagon response over time were significantly different than those observed in the saline control group. As expected, plasma GLP-1 levels were significantly higher in the GLP-1 treatment group compared to the saline controls (Table 1). Myocardial glucose uptake was also greater in the GLP-1 treated rats and the response was significantly greater than that observed in the saline control (Table 2).

LV hemodynamic parameters

At baseline, there was no significant difference between groups in mean arterial pressure, heart rate, stroke volume, LV dimensions or LV ejection fraction (Table 3). Notably, both groups had significant baseline hypertension with mean arterial pressures in excess of 130 mmHg.

Following the 3-month treatment period, significant differences were observed between the groups. The saline control group demonstrated decreased stroke volume and LV ejection fraction and increased LV end-systolic dimension over three months. By contrast, cardiac output *increased* in the GLP-1 treated group, and LV end-systolic dimensions, LV ejection

fraction, and heart rate remained unchanged (Table 3). These findings were corroborated by *in vitro* data, which showed that LV systolic pressure and LV developed pressures were significantly greater in the GLP-1 treated rats (Table 2). The decline in LV systolic and developed pressure in the isolated hearts of the saline treated group reflects a decline in LV contractile performance under isovolumic conditions. Thus, GLP-1 treatment preserved LV size and function, preventing the deterioration observed in the saline control group.

Survival

SHHF rats treated with GLP-1 had significantly greater survival (72%) after three months compared with saline controls (44%) (Figure 1). This was associated with significantly reduced myocyte apoptosis as assessed by Tunel staining. Notably, there was no difference in apoptotic cell death among non-myocyte cells in either treatment group (Figure 2). The reduction in myocyte apoptosis in the GLP-1 treatment group was accompanied by reduced myocardial caspase-3 activation (Figure 3). In addition, GLP-1 treated rats had increased Akt phosphorylation and GLUT-4 translocation, consistent with the increase in plasma insulin. However, Akt activation was not associated with inactivation of the pro-apoptotic protein BAD as assessed by the ratio of ser¹³⁶ p-BAD / total BAD (Con: 0.49±0.03; GLP-1: 0.45±0.03). GLP-1 receptor (GLP-1R) expression was increased in GLP-1 treated rats (Figure 4).

Discussion

Obesity, hypertension, and diabetes are well-established risk factors for cardiovascular mortality in humans, but strategies designed to modify these risks have been largely elusive. In the present study, we demonstrate that a 3-month, continuous infusion of GLP-1 is associated with improvements in myocardial functional and systemic metabolic parameters, as well as with prolonged survival in an obese, hypertensive rat model predisposed to accelerated cardiovascular disease and premature mortality. To our knowledge, there are no previous studies of the effects of GLP-1 on cardiovascular mortality in this type of high-risk animal model.

The current observations are consistent with our prior study in human heart failure subjects, where a 5-week infusion of GLP-1 led to improvements in LV function, functional capacity, and systemic metabolic parameters⁴. In both the current and referenced study, better glycemic control in the GLP-1-treated group may have contributed to improvement in cardiac function, since elevated glucose levels have been shown to depress LV function in both *in-vitro*^{19,20} and *in-vivo* studies of normal subjects and diabetics^{21,22}. However, *non-diabetic* human subjects demonstrated similar improvements in myocardial function as diabetics, suggesting an effect of GLP-1 that is *independent* of glycemic control. Nevertheless, the inclusion of a positive control group receiving an anti-hyperglycemic agent other than GLP-1 would be required to clarify this issue further, although is beyond the scope of the present investigation.

This is also the first study to demonstrate the ability of GLP-1 to reduce cardiac myocyte apoptosis. Notably, reduced programmed cell death was not seen in non-myocyte cellular constituents. The ability of GLP-1 to protect cardiac myocytes from accelerated death was suggested in a study showing that infusion of GLP-1 is associated with improved left ventricular function in patients following successful reperfusion for myocardial infarction⁹. To date, the anti-apoptotic effects of GLP-1 have been examined most extensively in pancreas, where reduced programmed cell death has been observed in diabetic rodents, islet cell lines, purified rat β -cells, heterologous cells expressing the GLP-1 receptor, and human islet cells^{23,24,25,26,27}. Liraglutide, a long-acting GLP-1 analogue, has also been shown to inhibit apoptosis in primary neonatal rat islets²⁸. In addition to pancreatic cells, GLP-1 receptor activation enhances neuronal survival in cellular and animal models of neuronal toxicity^{29, 30}.

The mechanism of the anti-apoptotic action of GLP-1 remains the subject of intense investigation. Nevertheless, evidence has emerged for the involvement of increased phosphatidyl inositol-3 kinase (PI3K)/Akt signaling, leading to enhanced nuclear factor-kappa B DNA binding activity and stimulation of inhibitors of apoptosis protein-2, Bcl-XL and Bcl-2 expression^{24,25,26,31}. Activation of PI3K/Akt also functions to block BAD-mediated death by phosphorylating BAD at Ser-136, and increased levels of inactivated p-BAD have been demonstrated in rat hearts in response to treatment with GLP-1⁶. In the present study, we observed increases in phosphorylation of Akt associated with reduced levels of the activated form of caspase-3. However, protein levels of pBAD (ser 136) were not different between the GLP-1 treated and control groups. Taken together, these data suggest that GLP-1 treatment favors survival through reduced apoptosis, but additional work will be required to determine the precise mechanism.

In addition to the reduction in myocyte apoptosis, treatment with GLP-1 was associated with increased LV mass index. The ability of the left ventricle to hypertrophy in the presence of increased afterload is an adaptive response that acts to normalize wall stress. As seen in the saline treated group, failure of the ventricle to hypertrophy adequately leads to ventricular dilation and dysfunction. It is noteworthy that GLP-1 is known to promote hyperplasia in pancreatic β -cells^{32,33}. Several potential mediators of growth signaling by GLP-1 have been proposed, including protein kinase C ζ ³², epidermal growth factor receptor and c-src³³, as well as PDX-1³⁴. The mouse model of GLP-1 receptor knock out was associated with modest cardiac phenotype characterized by decreased cardiac mass and impaired responses to superimposed metabolic stresses³⁷. Whether our observation of increased LV mass index is due to direct effects of GLP-1, or whether this is an indirect result of the increase in plasma insulin remains uncertain. Increased activation of Akt is known to stimulate cardiac hypertrophy through both the activation of the mTOR pathway³⁵ and through the inactivation of GSK-3 β ³⁶. The inclusion of a control group receiving an alternative insulin secretagogue may help elucidate this issue in future studies.

We have shown previously that GLP-1 treatment in normal isolated, isovolumic hearts has a distinctly different physiological and signaling profile compared to insulin, with stimulation of GLUT-1 translocation via a non-Akt dependent mechanism⁷. In the present study, GLP-1 infusion resulted in increased plasma insulin levels, and associated phosphorylation and activation of Akt leading to GLUT-4 translocation. *In vitro* experiments confirmed that this was associated with increased myocardial glucose uptake. These apparently discrepant findings in these two rat models are largely due to the fact that in the current experiments, the administration of GLP-1 occurred in the setting of hyperglycemia. Under these circumstances, the insulinotropic actions of GLP-1 to stimulate pancreatic insulin release predominate over the insulinomimetic effects seen in isolated normal rat hearts under euglycemic conditions.

We also observed that GLP-1 infusion was associated with increased adiponectin expression. Both of these pathways may have contributed to increased myocardial glucose uptake and normalization of plasma glucose. The relationship between adiponectin and LV mass is not completely elucidated, although epidemiologic data in humans^{38,39} and experimental data in mice⁴⁰ has suggested that adiponectin *inhibits* hypertrophic signaling in the myocardium in the setting of pressure overload. In this case, the inhibitory effect of increased adiponectin must have been outweighed by other, more potent, growth stimulating pathways.

GLP-1R expression was more abundant in cardiac myocytes from GLP-1 treated rats. Very little is known about the regulation of the GLP-1R in the heart, and existing data from other tissues do not provide insight into our findings. High glucose concentrations upregulate receptor expression in pancreatic islets^{41,42,43,44}, but serum glucose was *lower* in the GLP-1 treated group than in controls in the current study (as would be expected with GLP-1 treatment).

Leptin enhances GLP-1R expression in certain areas of the rat hypothalamus⁴³ but our GLP-1 and control rats had similar serum leptin levels. Increased GLP-1R expression cannot be explained by treatment with GLP-1 itself, as this was shown to *downregulate* the GLP-1R gene in a rat medullary thyroid carcinoma cell line⁴⁵. Additional studies will be needed to identify other determinants of GLP-1R expression that could explain our current observations.

GLP-1 (7-36) amide undergoes rapid enzymatic cleavage by the enzyme dipeptidyl peptidase-4 (DPP-4), resulting in the formation of GLP-1 (9-36), the predominant plasma metabolite. We showed previously in a canine model of pacing-induced cardiomyopathy that infusion of the metabolite GLP-1-(9-36) mimics the effects of GLP-1-(7-36) in stimulating myocardial glucose uptake and improving LV and systemic hemodynamics⁶. The relative contribution of each of these peptides to our current observations cannot be determined. Future studies using co-administration of GLP-1 (7-36) with a DPP-4 inhibitor or Exendin-4, which is resistant to degradation by DPP-4, are warranted.

In conclusion, a three-month continuous infusion of GLP-1 resulted in preservation of LV function and prolonged survival in an experimental animal model of obesity, insulin resistance, and hypertension that was otherwise predisposed to premature cardiac failure and death.

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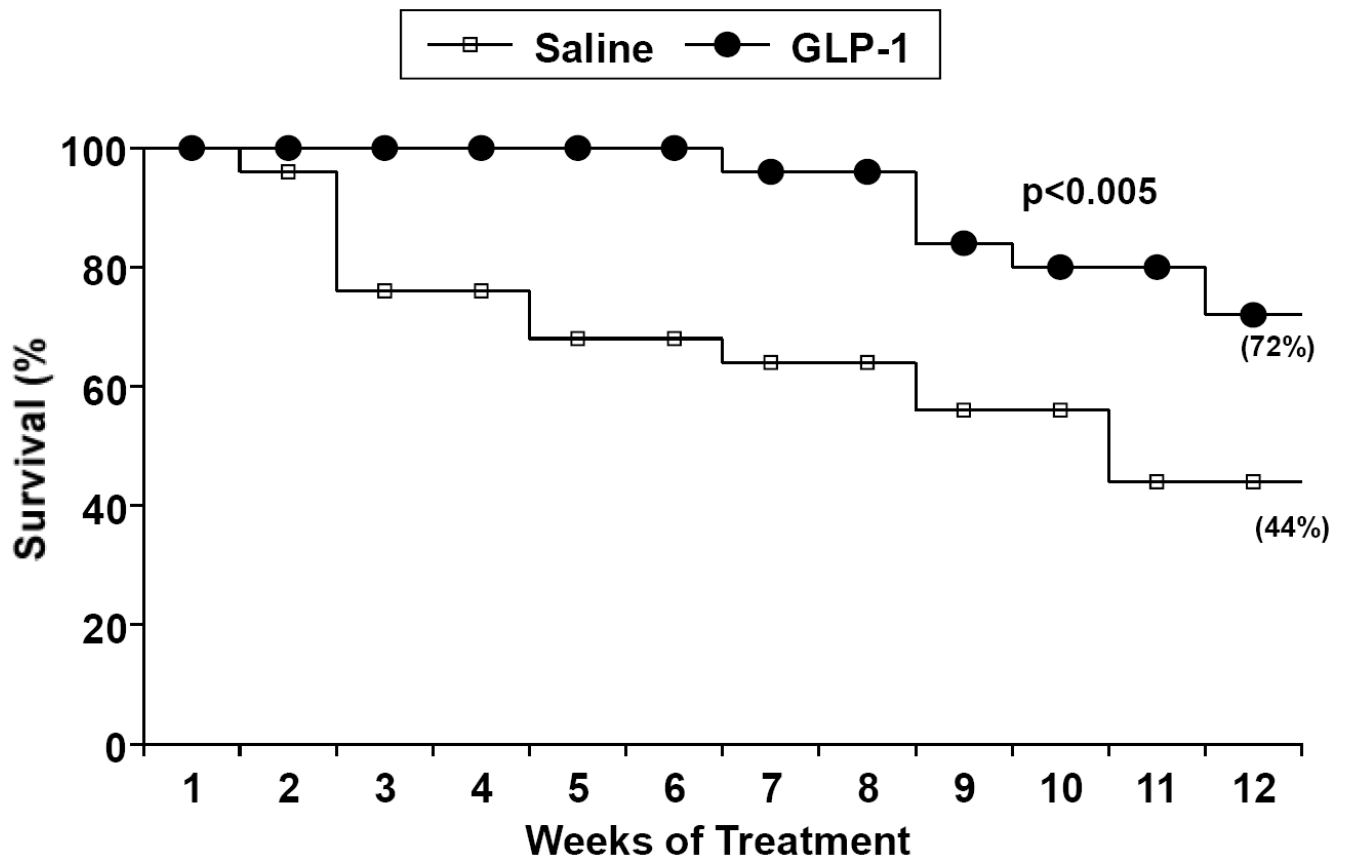


Figure 1. The survival of two groups of SHHF randomized to receive intraperitoneal continuous infusion of GLP-1 or saline. GLP treated animals had better survival compared to control ($p = 0.008$).

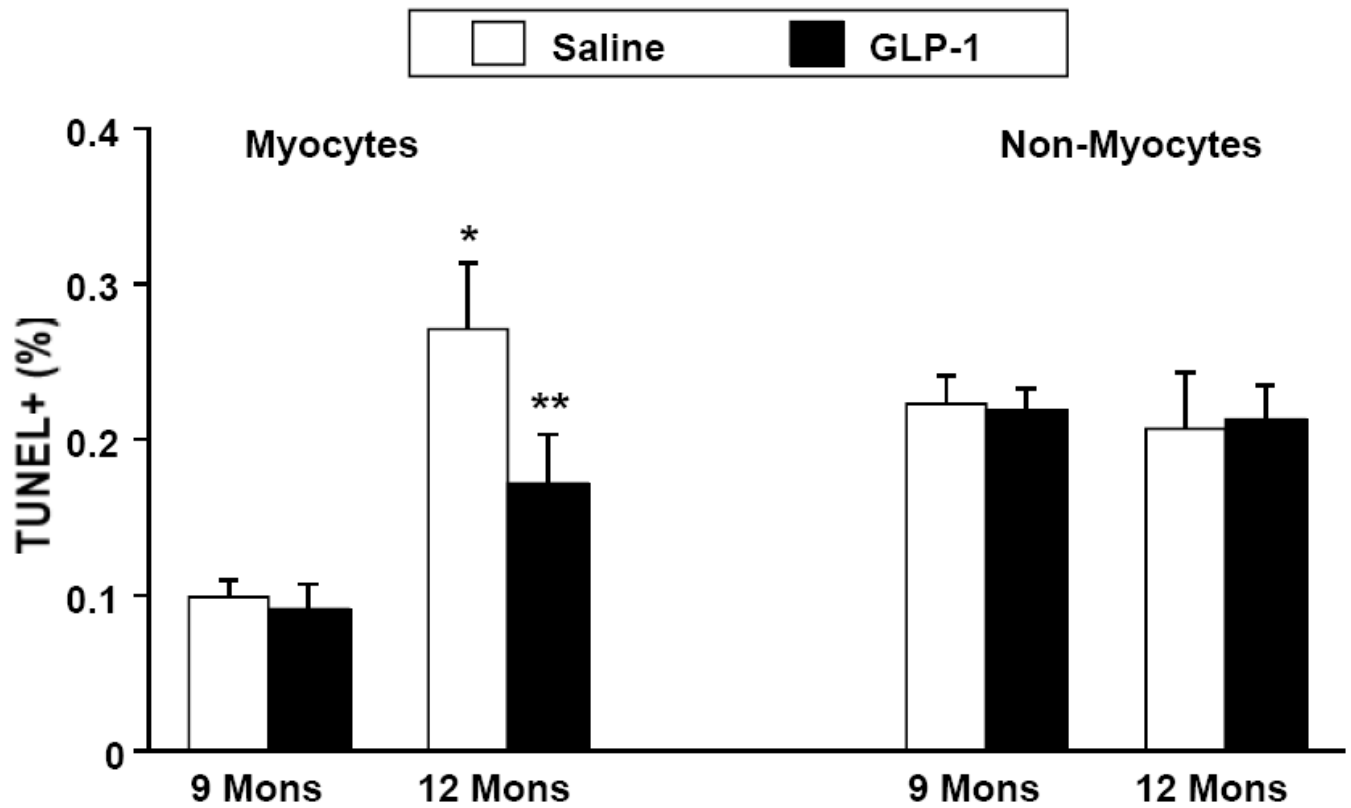


Figure 2.

Percent of myocyte and non myocyte cells staining positive for TUNEL at 9 months (n=5 in each group) and 12 months of in a group treated with GLP-1 (n=6) compared to saline control (n=5). There was significantly less myocyte apoptosis in the GLP-1 group at 12 months. There was no difference in non-myocyte apoptosis. *p = 0.0002 saline control at 9 versus 12 months. ** p = 0.03 GLP-1 compared to control at 12 months.

Caspase -3 Activation

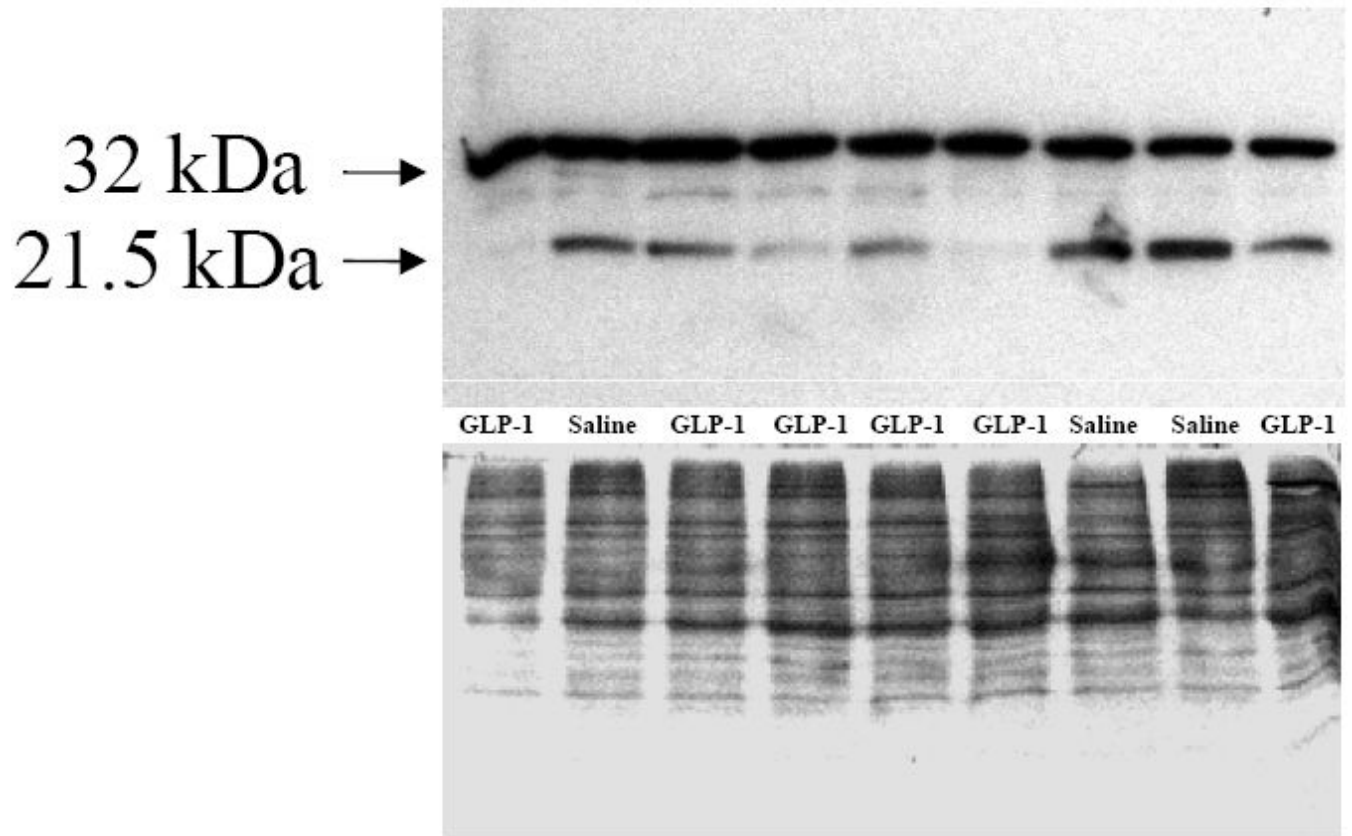


Figure 3. Western blots for caspase-3 protein (32 kDa) and activated, cleaved form of caspase-3 (21 kDa). GLP-1 treatment was associated with decreased caspase-3 activation.

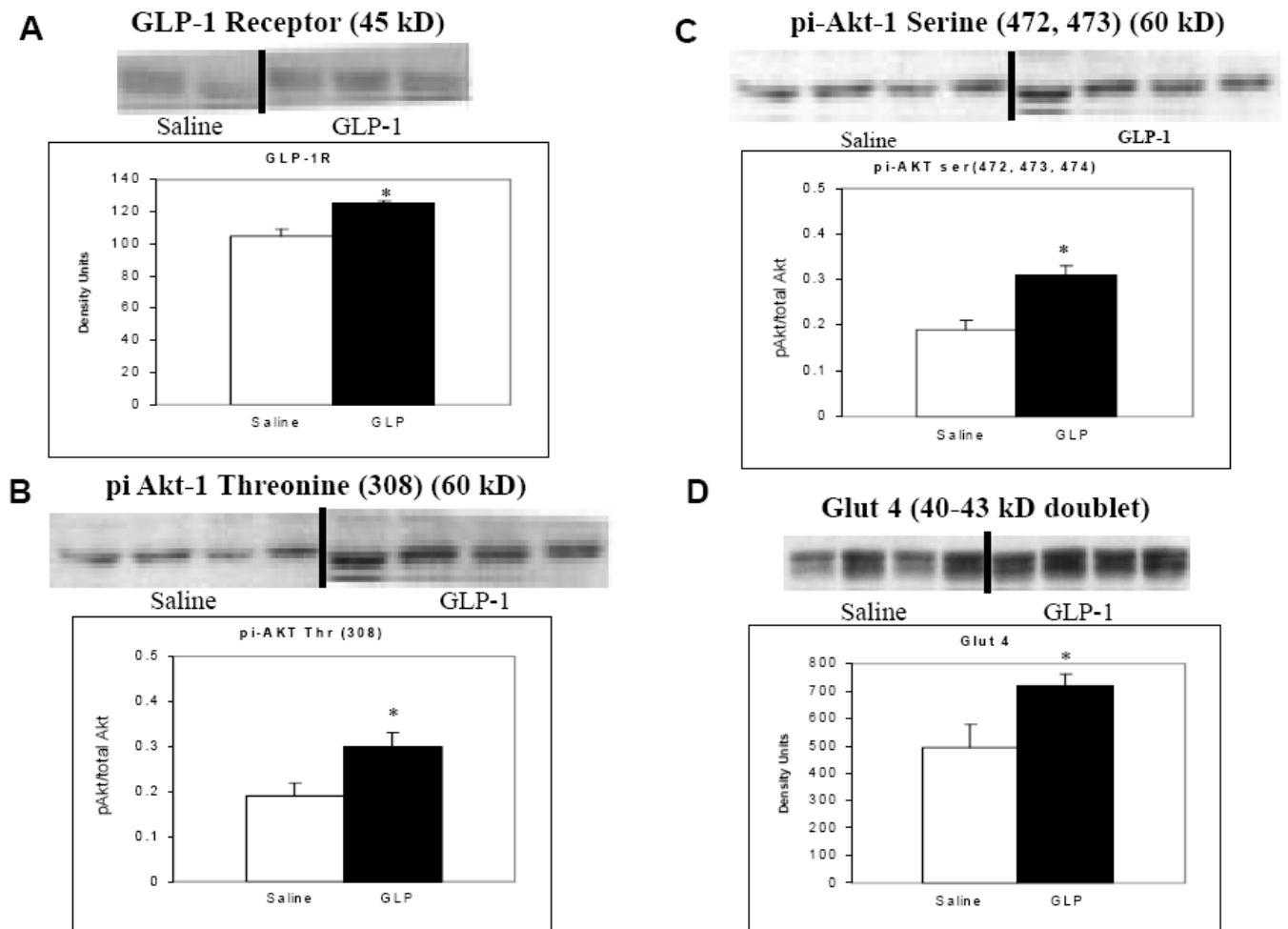


Figure 4.

The effects of GLP-1 on myocardial GLP-1 receptor expression (A), threonine phosphorylation of Akt (B), serine phosphorylation of Akt (C) and myocardial GLUT-4 translocation (D). GLP-1 receptor expression was upregulated in the GLP-1 treated group at 12 months ($p = 0.043$). There was increased Akt phosphorylation and activation in the GLP-1 treated group ($p=0.025$ for serine and $p = 0.018$ for threonine) with resultant increase in GLUT-4 translocation ($p = 0.043$).

Table 1

Morphometrics and Metabolic Parameters

	Saline Treatment		* p	GLP-1 Treatment		* p	† p
	n=10	n=11		n=10	n=18		
Age (months)	9	12		9	12		
Body Weight (gm)	733±16	731±21	0.94	744±19	641±35 ^{*†}	0.046	0.01
Body Mass Index (g/m ²)	11.6±0.3	11.4±0.7	0.80	11.7±0.3	9.7±0.5 ^{*†}	0.009	0.0001
LV mass (gm)	1.98±0.07	1.97±0.11	0.94	2.10±0.11	2.31±0.09	0.158	0.151
LV mass index (gm/m ²)	28±1	27±3	0.76	30±2	36±2 [†]	0.061	0.023
Glucose (mg/dl)	199±15	180±10	0.29	242±15	145±5 ^{*†}	0.0001	0.0001
NEFA (µmol/L)	523±105	587±61	0.59	541±61	585 ±58	0.630	0.558
Insulin (pmol/L)	1439±189	1679±210	0.049	1736 ±164	2309±178 ^{*†}	0.038	0.0001
Glucagon (pg/ml)	297±50	406±51 [*]	0.14	312 ±25	265 ±31 [†]	0.310	0.0001
Triglycerides (µmol/L)	1687±345	1567±213	0.77	1701±222	945±178 ^{*†}	0.032	0.0001
Adiponectin (µg/ml)	4.8±1.2	4.7±0.8	0.94	3.9±0.8	6.0±0.6 ^{*†}	0.044	0.0001
Leptin (ng/ml)	91±12	75±22	0.54	85±5	75 ±11	0.512	0.326
GLP-1 (pmol/L)	16±2	21±3	0.18	12±2	61±10 ^{*†}	0.001	0.001

All values expressed as mean±SEM.

* p value: 12 months compared to 9 months in each group

† p value: response over time in GLP-1 group compared to saline control

In-vitro Hemodynamic Parameters and Glucose Uptake: Isolated Perfused Heart Preparations

Table 2

	Saline Treatment		GLP-1 Treatment		* p	† p
	n=10	n=11	n=10	n=18		
Age (months)	9	12	9	12		
LV Systolic Pressure (mmHg)	136±6	114±7*	141±9	144±5 [†]	0.025	0.909
LV Developed Pressure (mmHg)	131±8	108±4*	138±10	137±10	0.011	0.936
LV (+)dP/dt (mmHg/sec)	3.471±261	3.312±213	3.399±136	3.491±136	0.638	0.754
LV (-) dP/dt (mmHg/sec)	2.401±161	1.512±113*	2.699±111	2.401±111 [†]	0.001	0.210
Heart Rate (min ⁻¹)	241±28	225±31	252±32	231±32	0.701	0.637
Coronary Flow (ml/mm)	26±2	24±1	25±2	24±2	0.647	0.627
Glucose Uptake (µm/ min/g)	1.7±0.06	1.3±0.05*	1.6±0.06	2.1±0.07 ^{**†}	0.0002	0.001

All values expressed as mean ± SEM.

* p value: 12 months compared to 9 months in each group

[†] p value: response over time in GLP-1 group compared to saline control

Table 3

In-vivo Hemodynamic Parameters

	Saline Treatment		GLP-1 Treatment		* p	† p
	n=10	n=11	n=10	n=18		
Age (months)	9	12	9	12		
Mean Arterial Pressure (mmHg)	133±16	127±21	141±19	134±15	0.826	0.776
Heart Rate (min-1)	376±28	401±27*	363±10	371±20	0.527	0.771
Stroke Volume (ml)	1.5±0.1	1.0±0.1*	1.3±0.1	1.4±0.1*†	0.001	0.497
Cardiac Output (ml/min)	564±38	402±43*	468±22	543±22*†	0.295	0.051
LV EDD (mm)	9.61±0.2	9.80±0.1	9.20±0.2	9.21±S†	0.357	0.944
LV ESD (mm)	1.73±0.23	2.94±0.18*	1.84±0.20	1.66 ±0.28	0.0004	0.631
LV Ejection (%)	82±4	70±4	80±4	82±3	0.033	0.661

All values expressed as mean±SEM.

* p value: 12 months compared to 9 months in each group

† p value: response over time in GLP-1 group compared to saline control