Published in final edited form as:
Basic Res Cardiol. 2013 July ; 108(4): 365. doi:10.1007/s00395-013-0365-x.

Impaired Cardiometabolic Responses to Glucagon-Like Peptide 1 in Obesity and Type 2 Diabetes Mellitus

Steven P Moberly[1], **Kieren J Mather**[2], **Zachary C Berwick**[1], **Meredith K Owen**[1], **Adam G Goodwill**[1], **Eli D Casalini**[1], **Gary D Hutchins**[3], **Mark A Green**[3], **Yen Ng**[3], **Robert V Considine**[1,2], **Kevin M Perry**[3], **Robin L Chisholm**[2], and **Johnathan D Tune**[1]

[1]Department of Cellular and Integrative Physiology, Indiana University School of Medicine, Indianapolis, Indiana

[2]Department of Medicine, Indiana University School of Medicine, Indianapolis, Indiana

[3]Department of Radiology and Imaging Sciences, Indiana University School of Medicine, Indianapolis, Indiana

Abstract

Glucagon-Like Peptide 1 (GLP-1) has insulin-like effects on myocardial glucose uptake which may contribute to its beneficial effects in the setting of myocardial ischemia. Whether these effects are different in the setting of obesity or type 2 diabetes (T2DM) requires investigation. We examined the cardiometabolic actions of GLP-1 (7–36) in lean and obese/T2DM humans, and in lean and obese Ossabaw swine. GLP-1 significantly augmented myocardial glucose uptake under resting conditions in lean humans, but this effect was impaired in T2DM. This observation was confirmed and extended in swine, where GLP-1 effects to augment myocardial glucose uptake during exercise were seen in lean but not in obese swine. GLP-1 did not increase myocardial oxygen consumption or blood flow in humans or in swine. Impaired myocardial responsiveness to GLP-1 in obesity was not associated with any apparent alterations in myocardial or coronary GLP1-R expression. No evidence for GLP-1 mediated activation of cAMP/PKA or AMPK signaling in lean or obese hearts was observed. GLP-1 treatment augmented p38-MAPK activity in lean, but not obese cardiac tissue. Taken together, these data provide novel evidence indicating that the cardiometabolic effects of GLP-1 are attenuated in obesity and T2DM, via mechanisms that may involve impaired p38-MAPK signaling.

Keywords

Obesity; diabetes; GLP-1; cardiac; glucose; metabolism

Introduction

Type 2 diabetes and pre-diabetic states of obesity and insulin resistance are associated with augmented cardiovascular risk [10, 19, 37], through mechanisms involving abnormal fuel

Correspondence: Johnathan D. Tune, Ph.D., Department of Cellular and Integrative Physiology, 635 Barnhill Drive, Indianapolis, IN 46202, Phone: 317-274-3433, Fax: 317-274-3318, jtune@iupui.edu.

Author contributions: SPM wrote the manuscript, researched data, and performed statistical analyses. ZCB, MKO, AGG, EDC, GDH, MAG, YN, RVC, KMP, and RLM researched data, reviewed the manuscript, and contributed to the discussion. KJM and JDT researched data, performed statistical analyses, and edited the manuscript. KJM and JDT take responsibility for the work as a whole, including study design, access to data, and decision to submit and publish the manuscript.

Conflict of Interest Disclosures
None

delivery and utilization, and vascular dysfunction [45]. Approaches to improve myocardial oxygen delivery and promote efficient fuel metabolism are currently being investigated as a means to improve the treatment of obesity- and diabetes-related cardiovascular disease [3, 17, 23, 24]. Emerging evidence suggests that glucagon-like peptide 1 (GLP-1) exerts cardioprotective effects, likely in part through its ability to augment myocardial glucose uptake [1, 4, 29, 46] and/or increase coronary blood flow [29]. Intravenous administration of GLP-1 significantly reduces myocardial infarct size following ischemia/reperfusion injury and improves cardiac contractile function in the setting of coronary artery disease, following myocardial ischemia, and in heart failure [4, 21, 25, 29–31, 39]. These data have been generated in non-diabetic, non-obese animal models, and relatively little is known about myocardial or vascular effects of GLP-1 in obesity or diabetes. In diabetic mice, the GLP-1 mimetic taspoglutide was not protective against atherosclerosis [32], and in Type 2 diabetic humans the GLP-1 mimetic exenatide failed to augment insulin-stimulated myocardial glucose uptake [11]. However, specific studies to delineate the influence of obesity and diabetes on the cardiovascular actions of GLP-1 are lacking.

We examined the effect of short-term, intravenous GLP-1 (7–36) administration on myocardial glucose uptake, myocardial perfusion, and cardiac function in lean and obese/T2DM humans using quantitative positron emission tomography (PET) and impedance cardiography. Further studies in lean and obese Ossabaw swine examined the cardiometabolic effects of intravenous GLP-1 (7–36) at rest and during exercise-induced increases in myocardial metabolism. Molecular studies in swine myocardial tissues evaluated the impact of obesity on GLP-1 receptor content and known GLP-1 signaling mediators including cyclic adenosine monophosphate (cAMP)/Protein Kinase A (PKA), p38 mitogen-activated protein kinase (MAPK) and AMP-activated protein kinase pathways [1, 2, 4, 5, 12, 42, 46].

Materials and Methods

Human subjects

The human studies protocols were approved by the Indiana University Institutional Review Board and conducted in accordance with the Declaration of Helsinki. Human subjects provided written informed consent. The human studies protocol was registered through ClinicalTrials.gov, (NCT01607450). The swine protocols performed were approved by the Indiana University Institutional Animal Care and Use Committee in accordance with the Guide for the Care and Use of Laboratory Animals.

Subjects between ages 18 and 50 years were studied. Lean control subjects had BMI <25 kg/m^2, and were in good general health, taking no regular medications. Obese type 2 diabetic subjects had BMI 30 to 40 kg/m^2, HbA1c 7.0 – 10.0%, and were treated with diet and exercise plus oral agents or insulin at the time of screening. Exclusion criteria at screening included: chronic illness (other than T2DM) or infection, known coronary artery disease or abnormal ECG at screening, blood pressure > 160/100 mmHg on two occasions during screening, total cholesterol >240 mg/dL, treatment with GLP-1 agonist or DPP-4 inhibitor within 6 months, treatment with PPAR-γ agonist within 6 months, recognized retinopathy, nephropathy, or neuropathy, unwillingness or inability to use injected insulin, inability to remain comfortably supine for the duration of the positron emission tomography (PET) protocol, and current pregnancy.

Preparation of human subjects with T2DM

The goal of diabetes management in the period leading up to PET measurements was to achieve dosing with basal insulin that would produce fasting glucose in the 90–120 mg/dL

range without requiring supplemental insulin on the morning of the PET study. Therefore, diabetic participants were placed on a uniform glucose management regimen using only insulin glargine (sanofi aventis) and lispro insulin (Eli Lilly and Co.) for two weeks prior to the scheduled PET study.

The day prior to PET studies, subjects ate only breakfast and then skipped the mid-day meal; that afternoon they were admitted to the Indiana CTSI Clinical Research Center for overnight GLP-1 infusion. Between 1800h and 2000h, pre-prandial 'pre-treatment' blood samples were collected and hemodynamic parameters were measured, then subjects used lispro insulin with a standardized meal (30% carbohydrates, 40% protein, 30% fat). Their standardized nighttime insulin glargine dosing was then administered to provide basal control of fasting sugar until completion of the PET protocol the following morning.

Human subjects study protocol

Human subjects were studied fasting, at rest. At the time of pre-prandial blood sampling the day prior to the PET studies, non-invasive measurements of hemodynamics were taken using surface electrodes placed on the thorax and neck to measure impedance (ICG, CardioDynamics). Beginning ~2200h subjects received an overnight intravenous infusion of normal saline (n = 6 lean subjects) or GLP-1(7–36) (1.5 pmol/kg/min, Bachem Inc; n = 8 lean subjects and n = 7 T2DM subjects). PET measurements began in the morning following this 10hr infusion, which was continued through the ~3 hr PET session. Following PET imaging, blood samples and hemodynamic measures were taken and test infusions were terminated.

PET Imaging

PET data were collected using a Siemens ECAT EXACT HR+ whole body PET scanner. ^{11}C and ^{18}F were generated using a Siemens medical cyclotron system. The infused compounds were then manufactured as previously described [13]. The image data were reconstructed using conventional filtered back-projection algorithms and a Hanning smoothing filter. A whole-heart volume of interest was generated for the set of images collected, and analyzed using computer-based parameter estimation algorithms, in order to extract kinetic parameters. Acetate data were metabolite corrected then modeled as a 2-compartment system allowing the derivation of total myocardial blood flow (MBF, derived from the rate of appearance of acetate in the myocardium) and total myocardial oxidation (MVO_2, derived from the rate of disappearance of acetate in the myocardium) [16, 27, 41]. Glucose data were modeled using the traditional 3-compartment approach [38] using kinetic parameters of tracer movement in and out of the myocardium to derive a quantitative measure of myocardial glucose uptake.

Ossabaw swine model of obesity

Lean control castrated male Ossabaw swine were fed ~2200 kcal/day of standard chow (5L80, Purina Test Diet) containing 18% kcal from protein, 71% kcal from complex carbohydrates, and 11% kcal from fat. Obese castrated male Ossabaw swine were fed an excess ~8000 kcal/day high fat, high fructose atherogenic diet containing 16% kcal from protein, 41% kcal from complex carbohydrates, 19% kcal from fructose, and 43% kcal from fat, and supplemented with 2.0% cholesterol and 0.7% sodium cholate by weight (KT324, Purina Test Diet). Swine were fed their respective diets for ~16 weeks prior to study procedures.

Swine surgical instrumentation

Through a left lateral thoracotomy performed under isoflurane anesthesia, a 17-Ga pressure-monitoring catheter (Edwards LifeSciences) was implanted in the descending thoracic aorta to measure aortic blood pressure and heart rate, and obtain arterial blood samples. Another catheter was placed in the anterior interventricular vein for coronary venous blood sampling from the LAD artery perfusion territory, and for intravenous drug administration. Patency of the coronary venous line was maintained by an elastomeric balloon pump (MILA International) which provided a continuous infusion of heparinized saline (5 U/ml at 5 ml/hr) into the catheter. The proximal LAD was secured to a perivascular flow transducer (Transonic Systems) for coronary blood flow measurements. Swine were allowed to recover 5–7 days prior to undergoing experimental protocols.

Swine experimental protocol

Preliminary studies demonstrated comparable augmentation of exercise-induced myocardial glucose uptake in lean animals following 10 hr vs 2 hr pre-treatment with GLP-1 (1.5 pmol/kg/min) and therefore swine studies used 2 hr exposures prior to the experimental protocol (~ 3 additional hours for protocol). Experiments were conducted at rest and during graded treadmill exercise, before and during the intravenous administration of GLP-1 (7–36). Each animal served as its own control. Aortic blood pressure, heart rate and LAD blood flow were continuously recorded at rest and during two levels of treadmill exercise (~2 mph and ~5 mph). Each exercise period lasted ~2 minutes. Swine were rested between bouts until hemodynamic parameters returned to baseline values. Arterial and anterior interventricular coronary venous samples were simultaneously collected at rest and during exercise. Blood samples were taken when hemodynamic variables had stabilized at each exercise level. These samples were analyzed for blood gases, oxygen content, lactate, and glucose (GEM Premier 3000 blood gas analyzer and 682 Co-oximeter system, Instrumentations Laboratories). Myocardial oxygen consumption (MVO_2) was calculated as coronary blood flow times the arterial coronary-venous difference in oxygen content. Myocardial glucose and lactate uptake were similarly calculated as coronary blood flow times the coronary-venous difference in glucose or lactate concentrations respectively. LAD perfusion territory was estimated to be 30% of total heart weight as previously described by Feigl [9].

Hormone and metabolite assays

In human samples, glucose was measured using a glucose oxidase method (YSI 2300 Plus, Yellow Springs Instruments) and free fatty acids were measured by colorimetric assay (Roche Diagnostics). Blood lipids were measured by the local hospital clinical laboratory. Insulin was measured by radioimmunoassay (HI-14K, Millipore-Linco). For humans and swine, total GLP-1 was measured by radioimmunoassay (GLP1T-36HK, Millipore-Linco; intra-assay CV 35% at 131 pmol/L; limit of detection 3 pmol/L). GLP-1 (7–36) was measured by ELISA (EGLP-35K, GLP1T-36HK, Millipore-Linco; intra-assay CV 6%, inter-assay CV 7% at 12 pmol/L; limit of detection 2 pmol/L).

Western blot analysis

Swine were allowed to recover from the exercise sessions for at least 48 hours before sacrifice. Following excision of the heart, the coronary circulation was perfused with physiologic salt solution to remove blood protein, then transmural left ventricular samples were quickly taken, frozen in liquid N_2 and stored at −80°C. These samples were later homogenized in lysis buffer, and protein was quantified using the DC protein assay (BioRad). 25 μg of protein was electrophoresed using 7% acrylamide gels, then transferred onto nitrocellulose membranes. Following overnight incubation with primary antibody at 4°C (Total p38-MAPK: #9218, Cell Signaling Technologies; phospho-p38 MAPK (Thr180/

Tyr182): #9216 Cell Signaling Technologies; GLP-1 receptor: ab39072, ABCAM, phospho-AMPKα (Thr172) #2531, Cell Signaling Technologies; AMPKβ1 #4178, Cell Signaling Technologies), membranes were exposed to secondary antibodies for 1 hr at ambient temperature. Band intensity was normalized to that of α-actin (691002, MP Biomedicals), and band molecular weight was determined using a standard protein ladder (161–0375, BioRad).

Immunohistochemistry and confocal microscopy

Frozen left ventricle samples were embedded in OCT (Tissue-Tek) and sliced into 10 μm sections. Slices were incubated in mouse monoclonal antibodies against cardiac troponin I (ab10231; 1:1,000), DAPI (100 ng/ml) and rabbit polyclonal antibodies raised against GLP-1R (ab39072; 1:25) for 1 hr. Slices were subsequently washed and treated with anti-rabbit IgG and anti-mouse IgG secondary antibodies for 30 min, conjugated with alexa−488 and alexa−594 respectively. Slides were imaged at 20X on an Olympus2 single photon confocal microscope.

Enzyme activity assays

Fresh transmural LV tissue slices (~200 μm thick) from lean and obese Ossabaw swine hearts were placed into preheated (37°C) tissue baths of physiological salt solution and oxygenated with 95% O_2 and 5% CO_2. The baths were then dosed with GLP-1 (7–36) to bring the concentration to 1 nM or 5 nM, or not dosed for a sham control. The tissue slices were incubated for 1 hr, then quickly rinsed and snap frozen in liquid N_2. PKA enzyme activity assays used the MESACUP® Protein Kinase Assay Kit (Code No. 5230; Medical and Biological Laboratories). p38-MAP Kinase enzyme activity was determined using a commercial assay (#9820, Cell Signaling Technologies). Western blots were also performed on sham and GLP-1 treated tissues slices to assess the ratio of phospho-AMPKα (Thr172) to (total) AMPKβi. *In vitro* measurements were performed in duplicate.

Statistical analysis

Population descriptive data are presented as mean ± SD; other data are presented as mean ± SE. For all statistical comparisons, a two-tailed $P<0.05$ was considered statistically significant. If normally distributed, statistical comparisons were made with one-way analysis of variance (ANOVA) or two-way ANOVA (Factor A: GLP-1 effect; Factor B: obesity effect) as appropriate. Human PET data were not normally distributed, and therefore we used a Kruskall-Wallace 1-way ANOVA, followed with Mann-Whitney U test for pairwise comparisons. When significance was found with parametric ANOVA, a Student-Newman-Keuls multiple comparison test was performed to identify differences between groups and treatment levels. Multiple linear regression analysis was used to compare slopes of variables plotted against MVO_2.

Results

Human Studies

Sex distribution and age were not different between lean and obese/T2DM human subjects (Table 1). Body mass index (BMI) was significantly elevated in obese/T2DM subjects. Baseline data were collected as pre-prandial samples following a skipped mid-day meal, at the time of admission to the Clinical Research Center the evening prior to study, and reflect metabolic standardization undertaken in diabetic participants prior to study. Under these conditions, plasma insulin and glucose were significantly elevated in the obese/T2DM group; non-esterified fatty acids were not different between groups (Table 1).

Pre-prandial plasma concentrations of total GLP-1 were significantly greater in obese/T2DM subjects. GLP-1 (7–36) infusion increased total plasma GLP-1 concentrations by 3 to 6-fold, and increased GLP-1 (7–36) concentrations by ~15 fold in both groups. These steady state concentrations achieved with GLP-1 infusion were ~4-fold higher than typical peak postprandial concentrations [43], but were not different between groups. By design, overnight GLP-1 plus basal insulin produced normal-range fasting plasma glucose in obese/T2DM subjects in preparation for the PET scanning procedure (Table 1). Non-esterified fatty acid concentrations following overnight treatment were not different across the 3 groups. Insulin concentrations under these normoglycemic conditions were within usual fasting ranges but differed across groups (Table 1). Glucagon concentrations were unaffected by obese/T2DM, and fell in all subjects following the overnight GLP-1 infusion ($P = 0.001$; Table 1).

At the time of admission, obese/T2DM subjects had higher systolic, diastolic and mean blood pressures, higher heart rates, and lower stroke volume compared to the lean subjects. Overnight GLP-1 administration did not significantly affect any hemodynamic parameter measured in lean or obese/T2DM human subjects, and at the time of PET study there were persisting elevations in blood pressures and heart rate in obese/T2DM versus lean subjects, although the stroke volume was no longer statistically different between groups (Table 1). At the time of PET study there were no significant differences in cardiac output or cardiac index across groups.

PET measurements

GLP-1 (7–36) increased resting myocardial glucose uptake by 2.8-fold in lean subjects, compared to untreated lean controls. In contrast, GLP-1-stimulated rates of myocardial glucose uptake in obese/T2DM were low and similar to saline control conditions in lean subjects (Figures 1A and 1B). Importantly, this blunted effect of GLP-1 on myocardial glucose uptake in obese/T2DM occurred despite similar plasma concentrations of GLP-1 (total and 7–36), similar concentrations of glucose and non-esterified fatty acids, and despite higher circulating insulin concentrations in obese/T2DM subjects (Table 1). Administration of GLP-1 did not significantly affect basal myocardial perfusion or MVO_2 in either group (Figures 1C and 1D).

Swine Studies at rest and during exercise

High-fat fed swine had significantly elevated body weight, mean aortic pressure, triglycerides, total cholesterol and ratio of LDL/HDL cholesterol (see Tables 2 and 3). Fasting levels of total GLP-1 and GLP-1 (7–36) were not different between lean and obese swine, and the plasma concentrations of GLP-1 (total and 7–36) increased similarly in both groups with GLP-1 (7–36) administration (Table 2). Under these euglycemic conditions, GLP-1 infusion had no effect on plasma insulin, glucose or lipids in the lean or obese swine.

Hemodynamic data showing effects of exercise with and without GLP-1 treatment are presented in Table 3. Administration of GLP-1 had no effect on resting mean blood pressure or heart rate, and did not modulate the exercise-induced increase in these parameters. MVO_2 was not different between lean and obese swine at rest or during exercise, and GLP-1 had no significant effect on these measures in either group (Table 3). GLP-1 tended to increase myocardial blood flow by exercise stage in lean versus obese swine ($P = 0.07$ for group difference; Table 3). However, this trend is likely related to the significant reduction in hemoglobin concentration noted in lean swine following administration of GLP-1 ($P = 0.02$; Table 3).

In lean swine, GLP-1 significantly increased the slope of the relationship between myocardial glucose uptake and MVO_2 during exercise (Figure 2A). In obese swine, no such increase was seen and in fact the association between myocardial glucose uptake and MVO_2 was lost (Figure 2B). Resting arterial lactate concentration was significantly elevated in obese swine compared with lean swine (Table 2), and myocardial lactate uptake increased as a function of MVO_2 in both lean and obese swine; however, GLP-1 had no effect on this relationship (Figures 2C and 2D). Linear regression analysis of myocardial oxygen delivery vs. MVO_2 demonstrated no effect of GLP-1 on the balance between coronary blood flow and myocardial metabolism in lean or obese swine (Figures 2E and 2F).

Myocardial Tissue Studies

Immunohistochemistry with confocal fluorescence microscopy revealed myocardial and trans-adventitial coronary artery expression of GLP-1R in swine (Figures 3B and 3C). A representative Western blot demonstrating selectivity of the GLP-1R antibody across species (swine, canine) is shown in Figure 3A. Densitometric analysis revealed no differences between lean and obese swine in the total coronary or crude cardiac GLP-1R content (Figure 3D).

In untreated (sham control) cardiac tissue from lean swine, cAMP increased PKA activity ~25-fold (Figure 3E). Basal and cAMP-stimulated PKA activities were similar in cardiac tissue from lean and obese swine. However, treatment of cardiac tissue slices with GLP-1 (7–36) did not alter untreated or cAMP-stimulated PKA activity in samples from lean or obese swine (Figure 3E). Total p38α-MAPK content was not different in cardiac tissue from lean or obese swine (Figures 4A, 4B). Additional Western analysis also showed that the amount of phospho-p38 MAPK (Thr180/Tyr182) was not different between lean (100 ± 15 % of lean sham) and obese (93 ± 17 % of lean sham) swine under untreated ($P = 0.84$) or 5 nM GLP-1 treated ($P = 0.62$) conditions (Figure 4D). Enzymatic activity of p38MAPK in cardiac tissue from lean and obese swine treated with saline or GLP-1 (7–36) was evaluated as production of the p38-MAPK product phosopho-ATF-2 (Figure 4E). p38MAPK activity increased significantly in response to 5 nM GLP-1 administration in cardiac tissue from lean swine ($P = 0.04$). In contrast, and despite comparable total p38MAPK content and phospho-p38MAPK content, p38MAPK activity was markedly diminished in hearts from obese swine under control conditions and was unchanged by GLP-1 administration (Figure 4F). GLP-1 treatment did not result in significant activation of myocardial AMPK as assessed by the ratio of phospho-AMPKα (Thr172) to (total) AMPKβ1 in lean ($P = 0.57$) or obese ($P = 0.89$) swine (Figure 5A, 5B).

Discussion

We report the first observations of impaired effects of GLP-1 (7–36) to stimulate myocardial glucose uptake in obese/T2DM humans compared to lean humans, and in obese versus lean Ossabaw swine and explored the mechanisms underlying these changes in lean and obese Ossabaw swine. Systemic infusion of GLP-1(7–36) augmented myocardial glucose uptake under resting conditions and during exercise in lean but not in obesity/T2DM. GLP-1 did not increase cardiac work, or act as a vasodilator in either species. Impaired myocardial responsiveness to GLP-1 in obesity was not associated with alterations in myocardial or coronary GLP1-R expression. Despite evidence that GLP-1 signals are mediated by cAMP/PKA pathways in other tissues, we found that GLP-1 did not activate cAMP/PKA or AMPK signaling in lean or obese hearts. GLP-1 treatment augmented myocardial p38-MAPK activity in lean, but not obese cardiac tissue. Taken together, these data indicate that the cardiovascular effects of GLP-1 are attenuated in obesity and T2DM, via a mechanism that may involve impaired stimulation of p38-MAPK signaling.

Cardiometabolic and hemodynamic effects of GLP-1 in obesity/T2DM

A significant novel finding of this study is significant attenuation of GLP-1-mediated increases in myocardial glucose uptake observed in under resting conditions in T2DM versus lean humans and under exercise conditions in obese versus lean Ossabaw swine (Figures 1 and 2). Our findings indicate that the defect in GLP-1 stimulated myocardial glucose uptake in obesity/T2DM is related to an impairment of myocardial GLP-1 responsiveness, since plasma GLP-1 concentrations were not different between lean and obese/T2DM groups within each species. Following the GLP-1 infusion, insulin and glucagon were higher in obese/T2DM subjects than controls, but these differences would predispose to elevated glucose uptake in the diabetes subjects and therefore do not confound the interpretation of impaired GLP-1 response. Differences in fuel availability also do not explain the observed defect, since by design there were no group differences in concentrations of glucose and non-esterified fatty acids at the time of measurement in humans (Table 1) and in swine (Table 2). It is possible that even with low fatty acid concentrations, differential rates of fatty acid uptake contribute to differences in fuel selection between obese/T2DM and lean phenotypes [34], but we did not directly measure rates of fatty acid uptake in the current studies. The swine data suggest that the observed impairment in GLP-1 effect on myocardial fuel selection is present in obesity without concurrent hyperglycemia, but from the current data we cannot determine whether there are additive effects of hyperglycemia, insulin resistance, or diabetes on top of the effect of obesity.

In this study of otherwise healthy lean and obese/T2DM humans, heart rate, blood pressure, myocardial perfusion, cardiac output, and stroke volume were not affected by systemic GLP-1 administration (Table 1). This was also true overall for the swine: GLP-1 did not affect heart rate or mean blood pressure in lean or obese swine, and although we observed a significant effect on diastolic blood pressure at the highest level of exercise ($P = 0.04$), the overall effect of exercise was not statistically modified by GLP-1 treatment ($P = 0.28$; Table 3). Numerous studies have shown that in the settings of heart failure or ischemia-reperfusion injury intravenous GLP-1 increases cardiac output, stroke volume, and other parameters of left ventricular performance [4, 21, 25, 29–31, 35, 39]. Our findings suggest that systemic exposure to GLP-1 does not affect hemodynamics or left ventricular performance in the non-injured, nonfailing heart.

Our studies in lean swine demonstrate that although GLP-1 modestly increases coronary blood flow in lean swine (Table 3), this effect is related to diminished hemoglobin concentration as GLP-1 administration had no effect on the relationship between myocardial oxygen delivery and metabolism in either lean (Figure 2E) or obese (Figure 2F) swine. Although vasodilator effects of GLP-1 have been reported in isolated rodent hearts [1, 46] and in conscious canines with pacing-induced dilated cardiomyopathy [29], other studies report no such effects [4, 7, 26, 30]. However, studies in humans with Type 2 diabetes have shown that the GLP-1 mimetic exenatide increased insulin-stimulated coronary blood flow [11]. These inconsistent findings point to the need for further studies of the coronary vascular effects of GLP-1 in the setting of obesity/T2DM.

Molecular mechanisms for impaired cardiometabolic effects of GLP-1 in obesity/T2DM

Receptors—To investigate the molecular mechanisms underlying the impaired responses to GLP-1 in obesity, we examined coronary and myocardial GLP-1R expression, and effects of GLP-1 on PKA activity, p38-MAPK activity, and activation of AMPK. We found no evidence for an obesity-associated defect in cardiac or coronary GLP-1R contents (Figure 3D). There is currently uncertainty about the specificity of commercially available antibodies against GLP-1R [32, 36] and variant forms of the GLP-1 receptor have been

previously reported [4], casting uncertainty on the interpretation of these observations. Nevertheless we detected only one molecular weight isoform of the GLP-1R using antibodies against the conventionally accepted receptor in swine at the correct molecular weight for a non-glycosylated variant. More importantly this antibody signal was not different between lean and obese swine hearts, indicating that differences in tissue content of the detected receptor do not explain the group differences in GLP-1 response.

Messengers—Previous studies suggest that the myocardial effects of GLP-1 are mediated via activation of two functionally distinct signaling pathways, namely the cAMP/PKA pathway [1, 6, 42] and the p38-MAPK signaling pathway [4, 46]. These pathways are distinct from those used by insulin to affect myocardia glucose uptake, and in particular there is no evidence that GLP-1 affects myocardial glucose uptake via Akt [4, 15, 46]. In contrast to prior reports in rodents, we found no evidence for an effect of GLP-1 on PKA activity in myocardium under basal or cAMP-stimulated conditions in swine (Figures 3E) suggesting that PKA is not a necessary component of the cardiac GLP-1 response. GLP-1 increased p38-MAPK activity in lean hearts (Figure 4C and 4D). Despite equal protein content in lean vs. obese hearts (Figure 4B), basal and GLP-1 stimulated p38-MAPK activity were markedly reduced in obese swine hearts (Figure 4F). These data implicate the p38-MAPK pathway in the cardiometabolic effects of GLP-1, rather than the cAMP/PKA pathway that mediates the effects of GLP-1 in islet cells [8, 14]. Previous investigations in rats and dogs have provided evidence that p38-MAPK mediates the effect of GLP-1 on myocardial glucose uptake [4, 46]. AMPK has been shown to regulate myocardial glucose uptake via recruitment and activation of p38-MAPK [20], and in other tissues AMPK appears to contribute to GLP-1 signaling [2, 12]. However, our *in vitro* experiments failed to detect significant GLP-1 induced activation/phosphorylation of AMPKα (Figure 5B). It is unknown whether similar defects are present in signaling responses to other beneficial factors that act via AMPK, such as adiponectin [44]. Taken together, these findings implicate diminished p38-MAPK activity (independent of total p38-MAPK expression or Thr180/Tyr182 phosphorylation) as a component of the impaired cardiometabolic responses to GLP-1 in the setting of obesity and T2DM.

Implications for clinical applications of GLP-1 for the heart

In experimental studies GLP-1 based therapies have been shown to provide protection from, and treatment of, cardiac injury/failure [1, 5, 25, 28, 29, 31, 39, 46]. The first clinical studies to apply GLP-1 for the treatment of ischemia suggest similar beneficial effects are possible in humans [22, 31], but dysglycemic patients have not been extensively evaluated to date. One proof-of-concept study suggested inotropic benefits in both diabetic and non-diabetic subjects with late-stage heart failure [39], but measures of myocardial glucose uptake were not obtained. Analogous to our main finding, a recent publication found no effect of exenatide (a GLP-1 mimetic) to augment insulin-stimulated myocardial glucose uptake in humans with Type 2 diabetes [11]. If the cardiac benefits of GLP-1 under ischemic conditions depend on changes in glucose uptake (a reasonable but untested assumption), our findings may herald impaired ischemic protection from GLP-1 in obese and T2DM populations. Also, there is evidence that inhibition of GLP-1 cleavage using DPP4 inhibitors may produce unintended changes in local vascular control of coagulation in coronary microvessels [18]. These observations suggest that GLP-1 family therapies may not confer the hoped-for cardiac benefits in diabetic patients.

The current data do not clearly identify the nature of the GLP-1 species that produced the observed effects on myocardial glucose uptake. Recent observations suggest that myocardial actions of GLP-1 are mediated at least in part by GLP-1(9–36), the product of GLP-1 cleavage by DPP4 [1, 30, 40]. In the current study the measured steady state concentrations

of GLP-1(9–36) did not differ between lean and obese subjects (Table 1), arguing against differential availability of this GLP-1 species as a contributor to the observed effects. Interestingly, recent efforts suggest benefits also with GLP-2, an alternate cleavage product of the preproglucagon gene [33], through mechanisms similar to those implicated in the anti-ischemic effects of GLP-1. This suggests that anti-ischemic effects may be obtained using a variety of GLP-1 family peptides. Future studies are needed to evaluate comparative cardiovascular actions of intact vs. cleaved GLP-1 or GLP-2 on myocardial blood flow, metabolism, contractile function and efficiency in the setting of obesity and/or T2DM.

Limitations of the investigation

Strengths of these observations include the direct demonstration of comparable responses in humans and in a swine model of metabolic and cardiac disease, combined with the use of swine tissue to explore mechanistic underpinnings of the impaired physiologic responses. Although the lack of a saline-treated human T2DM group is a limitation, the absence of this group does not prevent interpreting the available data, since even if there was a missed augmentation of myocardial glucose uptake by GLP-1 in T2DM (i.e. starting from a lower value than the observed readings with GLP-1), the low achieved myocardial glucose uptake level, numerically similar to untreated lean controls, is interpretable as a defect in absolute GLP-1 response in T2DM. The concordant swine data bolster this interpretation. Another limitation is the apparent inconsistency in effect on resting myocardial glucose uptake in swine vs. humans. Swine were pre-treated for 2 hours prior to measurement (vs. 10 hours for the human study protocol), and smaller increments in plasma GLP-1 concentration were achieved in swine relative to humans. These factors may explain the absence of an effect to increase myocardial glucose uptake at rest in the swine study protocol. Nevertheless, this GLP-1 exposure was sufficient to produce significant cardiac effects in lean swine under exercising conditions (Figure 2). The swine tissue studies were performed on tissues extracted well after recovery from the acute exercise study, avoiding confounding effects of exercise plus GLP-1 on the measured analytes and thus do not reflect the effects of the intravenous GLP-1 exposure. Regardless, the *in vitro* studies provide a more controlled and uniform exposure of myocardium to GLP-1 (7–36) that are directionally consistent with our *in vivo* findings. And finally, as noted above, although the current observations suggest that differences in myocardial expression of the GLP-1 receptor do not explain the observed differential responses, the available tools for immunologic detection of the GLP-1 receptor are currently being re-evaluated [4, 32, 36], which prevents definitive claims about the exact molecular species of GLP-1 receptor we detected. These observations will require confirmation as the available tools are improved.

Conclusions

Myocardial glucose uptake was increased in lean humans exposed to GLP-1 but this was not observed in obese/Type 2 diabetic humans. Similarly, effects of GLP-1 to augment exercise-induced myocardial glucose uptake and myocardial blood flow seen in lean swine were absent in obese swine. In parallel with impaired metabolic effects, actions of GLP-1 to augment p38MAPK activity seen in myocardial tissue from lean animals were absent in obese swine. These findings indicate that the cardiometabolic and hemodynamic actions of GLP-1 are impaired in obesity/Type 2 diabetes, possibly through a mechanism that includes impaired activation of p38MAPK.

Acknowledgments

This work was supported by NIH grants HL092245 (JDT), HL092799 (KJM), HL117620 (KJM and JDT), the Indiana Clinical and Translational Sciences Institute (TR000006) and the IU Medical Scientist Training Program (for SPM).

REFERENCES

1. Ban K, Noyan-Ashraf MH, Hoefer J, Bolz SS, Drucker DJ, Husain M. Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. Circulation. 2008; 117:2340–2350. [PubMed: 18427132]
2. Ben-Shlomo S, Zvibel I, Shnell M, Shlomai A, Chepurko E, Halpern Z, Barzilai N, Oren R, Fishman S. Glucagon-like peptide-1 reduces hepatic lipogenesis via activation of AMP-activated protein kinase. J Hepatol. 2011; 54:1214–1223. [PubMed: 21145820]
3. Berwick ZC, Dick GM, Tune JD. Heart of the matter: Coronary dysfunction in metabolic syndrome. J Mol Cell Cardiol. 2012; 52:848–856. [PubMed: 21767548]
4. Bhashyam S, Fields AV, Patterson B, Testani JM, Chen L, Shen YT, Shannon RP. Glucagon-like peptide-1 increases myocardial glucose uptake via p38alpha MAP kinase-mediated, nitric oxide-dependent mechanisms in conscious dogs with dilated cardiomyopathy. Circ Heart Fail. 2010; 3:512–521. [PubMed: 20466848]
5. Bose AK, Mocanu MM, Carr RD, Brand CL, Yellon DM. Glucagon-like peptide 1 can directly protect the heart against ischemia/reperfusion injury. Diabetes. 2005; 54:146–151. [PubMed: 15616022]
6. Bose AK, Mocanu MM, Carr RD, Yellon DM. Glucagon like peptide-1 is protective against myocardial ischemia/reperfusion injury when given either as a preconditioning mimetic or at reperfusion in an isolated rat heart model. Cardiovasc Drugs Ther. 2005; 19:9–11. [PubMed: 15883751]
7. Dokken BB, Hilwig WR, Teachey MK, Panchal RA, Hubner K, Allen D, Rogers DC, Kern KB. Glucagon-like peptide-1 (GLP-1) attenuates post-resuscitation myocardial microcirculatory dysfunction. Resuscitation. 2010; 81:755–760. [PubMed: 20347207]
8. Drucker DJ. The biology of incretin hormones. Cell Metab. 2006; 3:153–165. [PubMed: 16517403]
9. Feigl EO, Neat GW. Interrelations between coronary artery pressure, myocardial metabolism and coronary blood flow. J Mol Cell Cardiol. 1990; 22:375–390. [PubMed: 2388275]
10. Fox CS, Pencina MJ, Wilson PW, Paynter NP, Vasan RS, D'Agostino RB. Lifetime risk of cardiovascular disease among individuals with and without diabetes stratified by obesity status in the Framingham heart study. Diabetes Care. 2008; 31:1582–1584. [PubMed: 18458146]
11. Gejl M, Sondergaard HM, Stecher C, Bibby BM, Moller N, Botker HE, Hansen SB, Gjedde A, Rungby J, Brock B. Exenatide alters myocardial glucose transport and uptake depending on insulin resistance and increases myocardial blood flow in patients with type 2 diabetes. J Clin Endocrinol Metab. 2012; 97:E1165–E1169. [PubMed: 22544917]
12. Hattori Y, Jojima T, Tomizawa A, Satoh H, Hattori S, Kasai K, Hayashi T. A glucagon-like peptide-1 (GLP-1) analogue, liraglutide, upregulates nitric oxide production and exerts anti-inflammatory action in endothelial cells. Diabetologia. 2010; 53:2256–2263. [PubMed: 20593161]
13. Hicks RJ, Herman WH, Kalff V, Molina E, Wolfe ER, Hutchins G, Schwaiger M. Quantitative evaluation of regional substrate metabolism in the human heart by positron emission tomography. J Am Coll Cardiol. 1991; 18:101–111. [PubMed: 2050912]
14. Holst JJ. Implementation of GLP-1 based therapy of type 2 diabetes mellitus using DPP-IV inhibitors. Adv Exp Med Biol. 2003; 524:263–279. [PubMed: 12675249]
15. Huisamen B, Genade S, Lochner A. Signalling pathways activated by glucagon-like peptide-1 (7-36) amide in the rat heart and their role in protection against ischaemia. Cardiovasc J Afr. 2008; 19:77–83. [PubMed: 18516352]
16. Hutchins GD, Chen T, Carlson KA, Fain RL, Winkle W, Vavrek T, Mock BH, Zipes DP. PET imaging of oxidative metabolism abnormalities in sympathetically denervated canine myocardium. J Nucl Med. 1999; 40:846–853. [PubMed: 10319760]
17. Knudson JD, Dincer UD, Bratz IN, Sturek M, Dick GM, Tune JD. Mechanisms of coronary dysfunction in obesity and insulin resistance. Microcirculation. 2007; 14:317–338. [PubMed: 17613805]
18. Krijnen PA, Hahn NE, Kholova I, Baylan U, Sipkens JA, van Alphen FP, Vonk AB, Simsek S, Meischl C, Schalkwijk CG, van Buul JD, van Hinsbergh VW, Niessen HW. Loss of DPP4 activity

is related to a prothrombogenic status of endothelial cells: implications for the coronary microvasculature of myocardial infarction patients. Basic Res Cardiol. 2012; 107:233. [PubMed: 22167343]

19. Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, Salonen JT. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. JAMA. 2002; 288:2709–2716. [PubMed: 12460094]
20. Li J, Miller EJ, Ninomiya-Tsuji J, Russell RR 3rd. Young LH. AMP-activated protein kinase activates p38 mitogen-activated protein kinase by increasing recruitment of p38 MAPK to TAB1 in the ischemic heart. Circ Res. 2005; 97:872–879. [PubMed: 16179588]
21. Liu Q, Anderson C, Broyde A, Polizzi C, Fernandez R, Baron A, Parkes DG. Glucagon-like peptide-1 and the exenatide analogue AC3174 improve cardiac function, cardiac remodeling, and survival in rats with chronic heart failure. Cardiovasc diabetol. 2010; 9:76. [PubMed: 21080957]
22. Lonborg J, Kelbaek H, Vejlstrup N, Botker HE, Kim WY, Holmvang L, Jorgensen E, Helqvist S, Saunamaki K, Terkelsen CJ, Schoos MM, Kober L, Clemmensen P, Treiman M, Engstrom T. Exenatide reduces final infarct size in patients with ST-segment-elevation myocardial infarction and short-duration of ischemia. Circ Cardiovasc Interv. 2012; 5:288–295. [PubMed: 22496084]
23. Lopaschuk GD. Optimizing cardiac energy metabolism: how can fatty acid and carbohydrate metabolism be manipulated? Coron Artery Dis. 2001; 12(Suppl 1):S8–S11. [PubMed: 11286307]
24. Lopaschuk GD, Ussher JR, Folmes CD, Jaswal JS, Stanley WC. Myocardial fatty acid metabolism in health and disease. Physiol Rev. 2010; 90:207–258. [PubMed: 20086077]
25. Matsubara M, Kanemoto S, Leshnower BG, Albone EF, Hinmon R, Plappert T, Gorman JH 3rd, Gorman RC. Single dose GLP-1-Tf ameliorates myocardial ischemia/reperfusion injury. J Surg Res. 2011; 165:38–45. [PubMed: 19552923]
26. Moberly SP, Berwick ZC, Kohr M, Svendsen M, Mather KJ, Tune JD. Intracoronary glucagon-like peptide 1 preferentially augments glucose uptake in ischemic myocardium independent of changes in coronary flow. Exp Biol Med (Maywood). 2012; 237:334–342. [PubMed: 22345302]
27. Ng Y, Moberly SP, Mather KJ, Brown-Proctor C, Hutchins GD, Green MA. Equivalence of arterial and venous blood for [(11)C]CO(2)-metabolite analysis following intravenous administration of 1-[(11)C]acetate and 1-[(11)C]palmitate. Nucl Med Biol. 2013
28. Nikolaidis LA, Doverspike A, Hentosz T, Zourelias L, Shen YT, Elahi D, Shannon RP. Glucagon-like peptide-1 limits myocardial stunning following brief coronary occlusion and reperfusion in conscious canines. J Pharmacol Exp Ther. 2005; 312:303–308. [PubMed: 15356213]
29. Nikolaidis LA, Elahi D, Hentosz T, Doverspike A, Huerbin R, Zourelias L, Stolarski C, Shen YT, Shannon RP. Recombinant glucagon-like peptide-1 increases myocardial glucose uptake and improves left ventricular performance in conscious dogs with pacing-induced dilated cardiomyopathy. Circulation. 2004; 110:955–961. [PubMed: 15313949]
30. Nikolaidis LA, Elahi D, Shen YT, Shannon RP. Active metabolite of GLP-1 mediates myocardial glucose uptake and improves left ventricular performance in conscious dogs with dilated cardiomyopathy. Am J Physiol Heart Circ Physiol. 2005; 289:H2401–H2408. [PubMed: 16024574]
31. Nikolaidis LA, Mankad S, Sokos GG, Miske G, Shah A, Elahi D, Shannon RP. Effects of glucagon-like peptide-1 in patients with acute myocardial infarction and left ventricular dysfunction after successful reperfusion. Circulation. 2004; 109:962–965. [PubMed: 14981009]
32. Panjwani N, Mulvihill EE, Longuet C, Yusta B, Campbell JE, Brown TJ, Streutker C, Holland D, Cao X, Baggio LL, Drucker DJ. GLP-1 receptor activation indirectly reduces hepatic lipid accumulation but does not attenuate development of atherosclerosis in diabetic male ApoE(−/−) mice. Endocrinology. 2013; 154:127–139. [PubMed: 23183176]
33. Penna C, Pasqua T, Perrelli MG, Pagliaro P, Cerra MC, Angelone T. Postconditioning with glucagon like peptide-2 reduces ischemia/reperfusion injury in isolated rat hearts: role of survival kinases and mitochondrial KATP channels. Basic Res Cardiol. 2012; 107:272. [PubMed: 22699364]
34. Peterson LR, Herrero P, Schechtman KB, Racette SB, Waggoner AD, Kisrieva-Ware Z, Dence C, Klein S, Marsala J, Meyer T, Gropler RJ. Effect of obesity and insulin resistance on myocardial

substrate metabolism and efficiency in young women. Circulation. 2004; 109:2191–2196. [PubMed: 15123530]

35. Poornima I, Brown SB, Bhashyam S, Parikh P, Bolukoglu H, Shannon RP. Chronic glucagon-like peptide-1 infusion sustains left ventricular systolic function and prolongs survival in the spontaneously hypertensive, heart failure-prone rat. Circ Heart Fail. 2008; 1:153–160. [PubMed: 19727407]
36. Pyke C, Knudsen LB. The glucagon-like peptide-1 receptor--or not? Endocrinology. 2013; 154:4–8. [PubMed: 23267050]
37. Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Makuc DM, Marcus GM, Marelli A, Matchar DB, Moy CS, Mozaffarian D, Mussolino ME, Nichol G, Paynter NP, Soliman EZ, Sorlie PD, Sotoodehnia N, Turan TN, Virani SS, Wong ND, Woo D, Turner MB. Heart disease and stroke statistics--2012 update: a report from the American Heart Association. Circulation. 2012; 125:e2–e220. [PubMed: 22179539]
38. Schelbert, HR. Positron Emission Tomography of the Heart: Methodology, Findings in the Normal and Diseased Heart, and Clinical Applications. In: Phelps, ME., editor. PET: Molecular Imaging and its Biological Applications. New York: Spring-Verlag; 2004. p. 389-508.
39. Sokos GG, Nikolaidis LA, Mankad S, Elahi D, Shannon RP. Glucagon-like peptide-1 infusion improves left ventricular ejection fraction and functional status in patients with chronic heart failure. J Card Fail. 2006; 12:694–699. [PubMed: 17174230]
40. Sonne DP, Engstrom T, Treiman M. Protective effects of GLP-1 analogues exendin-4 and GLP-1(9–36) amide against ischemia-reperfusion injury in rat heart. Regul Pept. 2008; 146:243–249. [PubMed: 17976835]
41. Sun KT, Yeatman LA, Buxton DB, Chen K, Johnson JA, Huang SC, Kofoed KF, Weismueller S, Czernin J, Phelps ME, Schelbert HR. Simultaneous measurement of myocardial oxygen consumption and blood flow using [1-carbon-11]acetate. J Nucl Med. 1998; 39:272–280. [PubMed: 9476935]
42. Vila, Petroff MG.; Egan, JM.; Wang, X.; Sollott, SJ. Glucagon-like peptide-1 increases cAMP but fails to augment contraction in adult rat cardiac myocytes. Circ Res. 2001; 89:445–452. [PubMed: 11532906]
43. Vilsboll T, Krarup T, Deacon CF, Madsbad S, Holst JJ. Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. Diabetes. 2001; 50:609–613. [PubMed: 11246881]
44. Yan W, Zhang H, Liu P, Wang H, Liu J, Gao C, Liu Y, Lian K, Yang L, Sun L, Guo Y, Zhang L, Dong L, Lau WB, Gao E, Gao F, Xiong L, Qu Y, Tao L. Impaired mitochondrial biogenesis due to dysfunctional adiponectin-AMPK-PGC-1alpha signaling contributing to increased vulnerability in diabetic heart. Basic Res Cardiol. 2013; 108:329. [PubMed: 23460046]
45. Zhang H, Dellsperger KC, Zhang C. The link between metabolic abnormalities and endothelial dysfunction in type 2 diabetes: an update. Basic Res Cardiol. 2012; 107:237. [PubMed: 22189563]
46. Zhao T, Parikh P, Bhashyam S, Bolukoglu H, Poornima I, Shen YT, Shannon RP. Direct effects of glucagon-like peptide-1 on myocardial contractility and glucose uptake in normal and postischemic isolated rat hearts. J Pharmacol Exp Ther. 2006; 317:1106–1113. [PubMed: 16489128]

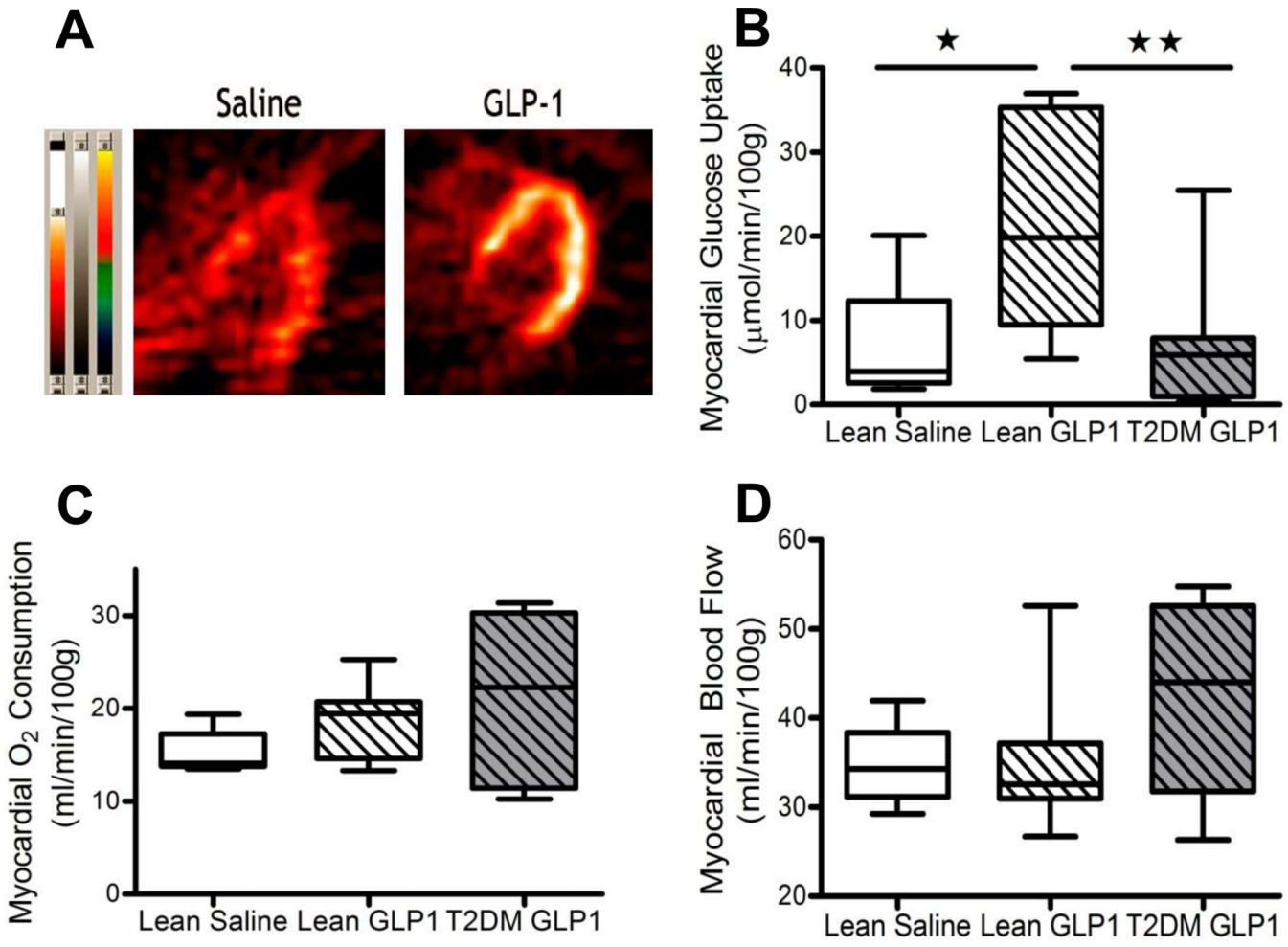

Figure 1.

(A) A representative PET image demonstrating the effect of GLP-1 on myocardial glucose uptake in lean human volunteers. (B) GLP-1 increased myocardial glucose uptake in the lean human heart but did not affect glucose uptake in obese T2DM humans. (C) Myocardial Oxygen Consumption was unaffected by GLP-1 administration in the lean human volunteers and in obese/T2DM (D) GLP-1 also did not significantly alter myocardial blood flow. Bars represent mean ± SE. (*) $P = 0.037$ vs. lean saline; (**) $P = 0.02$ vs. lean+GLP-1. Lean Saline (n = 6); Lean GLP-1 (n = 8); T2DM GLP1 (n = 7).

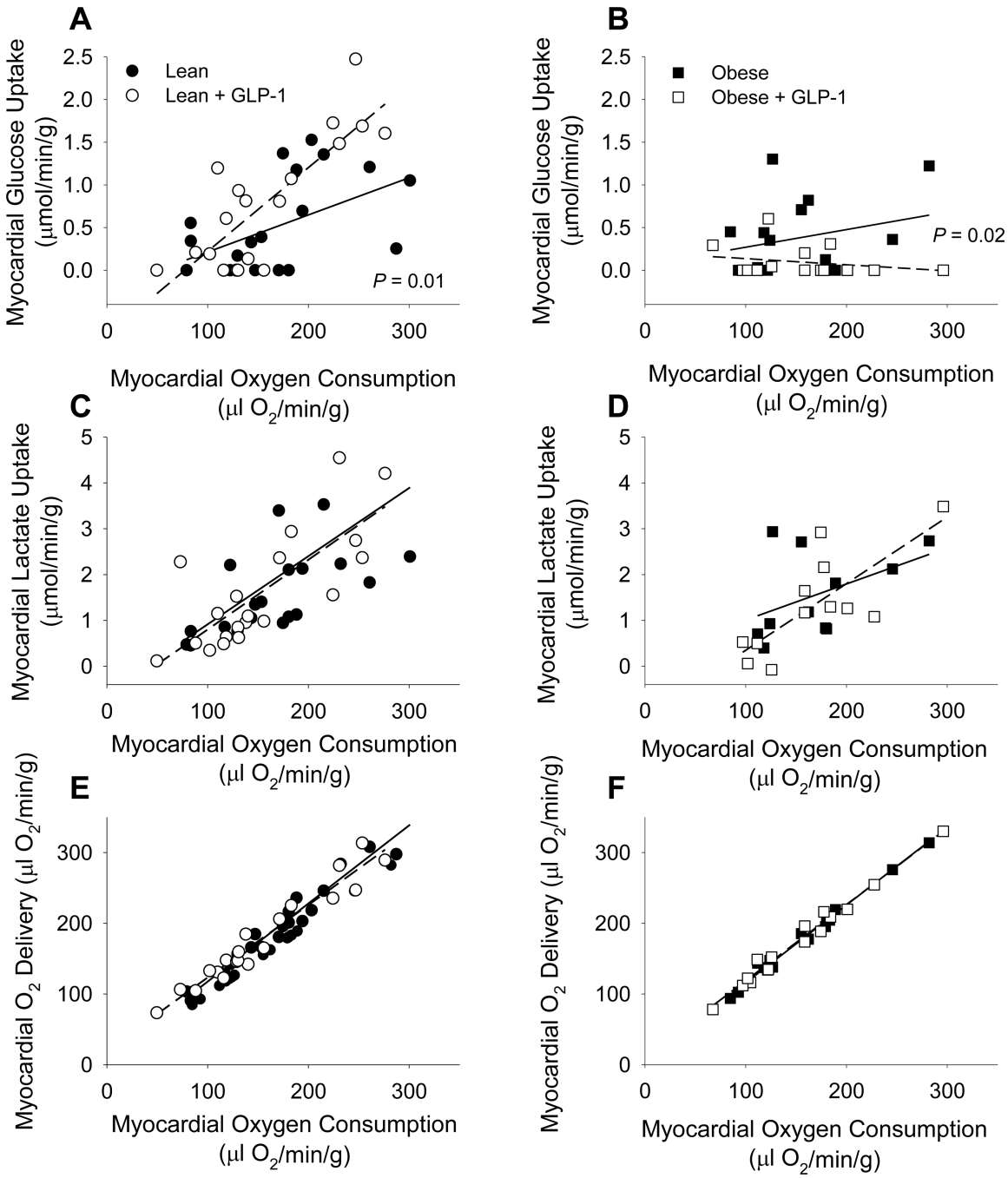

Figure 2.

GLP-1 increased myocardial glucose uptake in response to increasing myocardial oxygen consumption in exercising lean (n = 7) (A) but not obese (n = 5) (B) swine. Myocardial lactate uptake was not affected by GLP-1 administration in either lean (C) or obese (D) swine. GLP-1 also had no effect on the balance between myocardial oxygen delivery and metabolism in lean (E) or obese (F) swine.

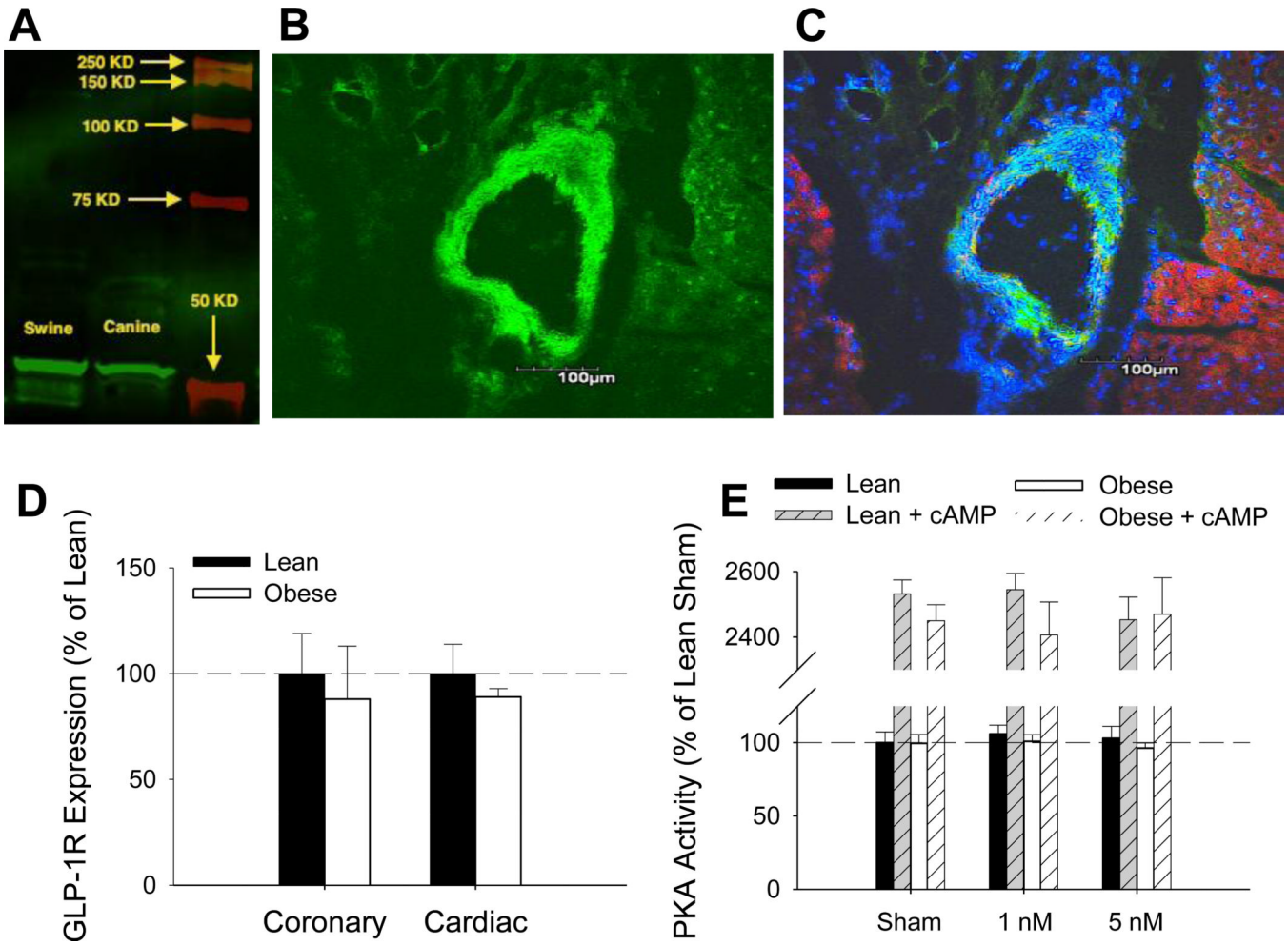

Figure 3.

(A) Representative gel demonstrating selectivity of GLP-1R antibody in cardiac extracts from Ossabaw swine and canines (canine data previously presented [26]). (B) GLP-1R (green) was present in the myocardium and coronary microvessels of swine. (C) Tissue architecture is further demonstrated with the nuclear stain DAPI (blue) and antibodies against cardiac troponin I (red). (D) Western Blot revealed no differences in GLP-1R content between lean (n = 4) and obese (n = 4) swine in either coronary or crude cardiac extracts. (E) Treatment of cardiac slices with GLP-1 (1 nM – 5 nM) for 1 hr had no effect on basal PKA activity in tissue from lean (n = 5) or obese (n = 5) swine. Addition of the PKA activator cAMP to the reaction mixture demonstrated a marked increase in PKA activity that was unaffected **by** GLP-1 treatment in cardiac tissue from lean or obese swine. Bars represent mean ± SE.

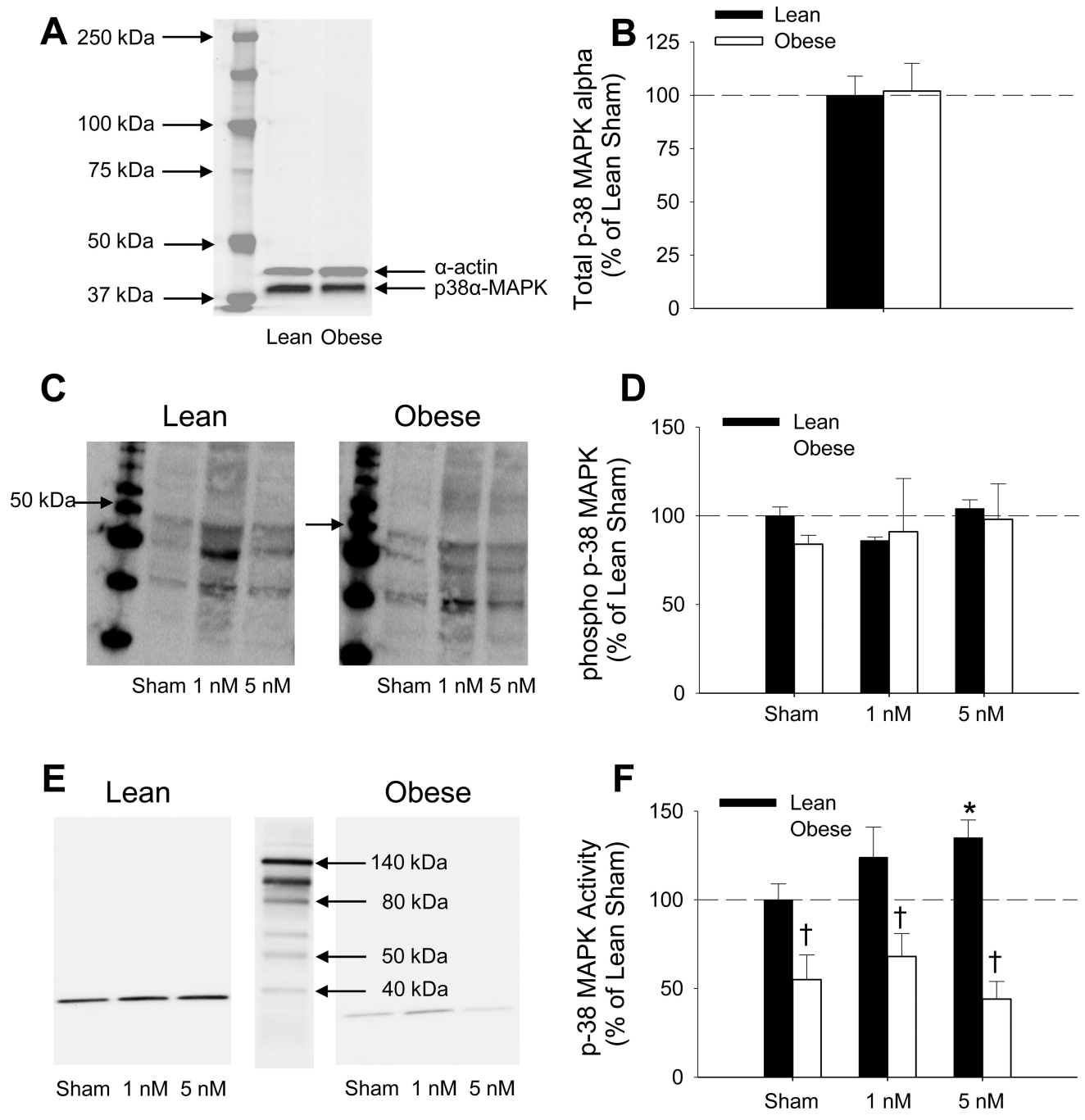

Figure 4.

(A) A representative image of total cardiac p38α-MAPK from lean and obese swine. (B) There was no difference in total cardiac expression of p38α-MAPK between lean (n = 4) and obese (n = 4) hearts. (C) A representative image of phospho p38 MAPK (Thr180/Tyr182) from lean and obese swine. (D) Treatment of cardiac slices with GLP-1 for 1 hr did not significantly alter phospho p38 levels in lean (n = 2) or obese (n = 2) swine. (E) Treatment of cardiac slices with GLP-1 (1nM - 5 nM) for 1 hr increased p38-MAPK activity in tissue from lean (n = 5) but not obese (n = 5) swine, and activity was lower in tissue from obese swine at all levels of treatment. (*) $P \leq 0.05$ vs. lean sham; (†) $P \leq 0.05$ vs. lean same condition.

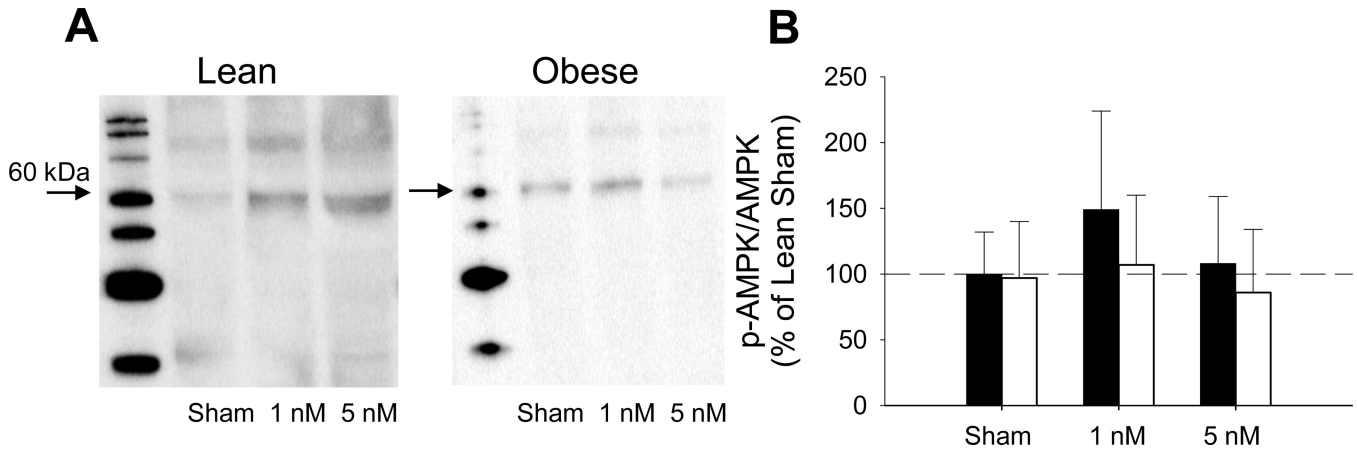

Figure 5.

(A) A representative image of phospho-AMPKα (Thr172) from lean (n = 3) and obese (n = 3) swine. (B) Treatment of cardiac slices with GLP-1 for 1 hr did not significantly increase AMPK activation in lean or obese swine (B). Bars represen* t mean ± SE.

Table 1

Participant characteristics, hemodynamic and metabolic parameters at the time of admission and following overnight infusion

	Lean – Saline		Lean – GLP-1		T2DM – GLP-1		Post Infusion Comparisons		
	Admission	**Post Infusion**	**Admission**	**Post Infusion**	**Admission**	**Post Infusion**	**Post Infusion 3 groups**	**Lean-Saline vs Lean-GLP-1**	**Lean-GLP-1 Vs T2DM-GLP-1**
Age (yrs	35.5 ± 10.6		46.0 ± 5.8		45.1 ± 7.9				
Sex (m/f)	1/5		3/5		1/6				
BMI (kg/m2)	23.9 ± 1.6		22.9 ± 1.8		30.3 ± 1.7*				
Percent Body Fat (%)	20.7 ± 9.1		21.1 ± 11.9		36.8 ± 6.7*				
HbA1c (%)	5.47 ± 0.40		5.56 ± 0.34		8.40 ± 3.02*				
Glucose (mmol/L)	5.1 ± 0.3	5.1 ± 0.6	5.1 ± 1.1	4.8 ± 0.3	6.7 ± 1.8*	5.4 ± 1.1	*P* = 0.08	*P* = 0.15	*P* = 0.055
NEFA (mmol/L)	0.13 ± 0.06	0.33 ± 0.03	0.19 ± 0.14	0.20 ± 0.10	0.20± 0.15	0.27 ± 0.18	*P* = 0.45	*P* = 0.13	*P* = 0.31
Insulin (μU/ml)	8.2 ± 3.2	6.9 ± 2.0	19.7 ± 6.3	10.4 ± 5.9	26.9 ± 13.9*	19.2 ±8.0	*P* = 0.001	*P* = 0.19	*P* = 0.01
Glucagon (pg/mL)	49.7 ± 12.5	41.0 ± 9.8	49.1 ± 9.9	41.7 ± 7.7	83.7 ± 12.5	62.7 ± 9.8	*P=0.20*	*P=0.98*	*P=0.10*
Total GLP-1 (pmol/L)	61.7 ± 33.5	38.2 ± 8.6	67.9 ± 34.6	302.1 ± 149.1	105.4 ± 48.8*	307.8 ± 86.2	*P* = 0.19	*P* = 0.001	*P* = 0.93
GLP-1(7–36) (pmol/L)		1.7 ± 1.2		72.0 ± 36.9	5.05 ± 2.50	81.1 ± 28.2	*P* = 0.10	*P* < 0.0001	*P* = 0.58
Systolic Pressure (mmHg)	112 ± 5	113± 10	117 ± 16	113± 14	134 ± 14*	139 ± 22	*P* = 0.001	*P* = 0.95	*P* = 0.004
Diastolic Pressure (mmHg)	70 ± 4	71 ± 8	64 ± 15	68 ± 9	78 ± 9*	83 ± 10	*P* = 0.002	*P* = 0.47	*P* = 0.004
Mean Pressure (mmHg)	85 ± 4	85 ± 8	82 ± 15	84 ± 10	97 ± 10*	104± 15	*P* < 0.001	*P* = 0.69	*P* = 0.002
Heart Rate (beats/min)	73.0 ± 12.5	66 ±9	69 ± 12	64 ±7	76 ±6*	72 ±6	*P* = 0.02	*P* = 0.62	*P* = 0.02
Cardiac Output (L/min)	5.87 ± 0.45	5.95 ±1.51	5.52 ± 1.34	5.44 ± 0.82	6.47 ± 2.35	6.07 ± 1.29	*P* = 0.36	*P* = 0.35	*P* = 0.20
Cardiac Index (L/min/m^2)	3.00 ± 027	3.00 ± 0.52	3.08 ± 0.68	2.88 ± 0.31	3.07 ± 0.99	2.90 ±0.51	*P* = 0.94	*P* = 0.52	*P* = 0.89
Stroke Volume (ml)	89.3 ± 6.1	92 ±22	82 ± 20	87 ±16	84 ±26	85 ±19	*P* = 0.67	*P* = 0.59	*P* = 0.82
Stroke Index (ml/m^2)	45.67 ± 4.51	46.3 ±9.2	45.4 ± 9.1	45.3 ±6.3	40.3 ±10.9*	40.4 ±7.5	*P* = 0.118	*P* = 0.77	*P* = 0.14

Values are mean ± SD for lean-saline (n = 6), lean-GLP-1 (n = 8), T2DM-GLP-1 (n = 7) human subjects.

*T2DM different from lean groups on admission.

Table 2

Phenotypic characteristics of lean and obese Ossabaw swine before and after GLP-1 GLP-1 (7–36) administration (1.5 pmol/kg/min, IV, 2 hrs).

	Lean	Lean + GLP-1	Obese	Obese + GLP-1
Body Weight (kg)	46.6 ± 2.3		69.0 ± 2.5 *	
Heart wt. / Body wt. (× 100)	0.37 ± 0.02		0.38 ± 0.01	
Glucose (mmol/L)	4.44 ± 0.28	4.17 ± 0.33	4.72 ± 0.28	4.17 ± 0.50
Insulin (pmol/L)	126 ± 30	120 ± 18	138 ± 18	108 ± 24
HOMA index	4.2 ± 1.0	3.7 ± 0.9	5.0 ± 0.9	3.6 ± 1.3
Total cholesterol (mmol/L)	2.3 ± 0.1	2.6 ± 0.2	12.4 ± 2.4 *	12.4 ± 2.5 *
LDL/HDL ratio	1.6 ± 0.1	1.7 ± 0.1	6.5 ± 1.9 *	4.3 ± 0.4 *
Triglycerides (mmol/L)	0.51 ± 0.06	0.45 ± 0.06	0.81 ± 0.15 *	0.94 ± 0.15 *
Lactate (mmol/L)	0.12 ± 0.01	0.11 ± 0.04	0.24 ± 0.08 *	0.20 ± 0.06
Total GLP-1 (pmol/L)	70 ± 18	104 ± 20	75 ± 15	112 ± 20
GLP-1 (7–36) (pmol/L)	8 ± 1	18 ± 4 *	8 ± 3	18 ± 7

Values are mean ± SE for lean (n = 7) and obese (n = 5) swine. Samples were obtained under resting conditions just prior to exercise protocol, following exposure to saline or GLP-1.

* $P \leq 0.05$ vs lean same condition.

Table 3

Hemodynamic variables at rest and during graded treadmill exercise in lean and obese Ossabaw swine with and without GLP-1 (7–36) (1.5 pmol/kg/min, IV, 2hrs

	Rest	Level 1	Level 2	GLP-1 Effect	Obesity	Interaction
Systolic Blood Pressure (mmHg)						
Lean	111 ±3	118 ± 3	119 ± 9	$P = 0.41$	$P = 0.01$	$P = 0.48$
Lean + GLP-1	117 ± 8	118 ± 7	126±6			
Obese	128 ± 7	125±4	134 ± 5	$P = 0.83$		
Obese + GLP-1	114 ± 7	125 ± 9	144 ± 9			
Diastolic Blood Pressure (mmHg)						
Lean	73 ± 3	79 ± 2	80 ± 3	$P = 0.65$	$P < 0.001$	$P = 0.28$
Lean + GLP-1	74 ± 5	77 ± 4	83 ± 5			
Obese	87 ± 7	80 ± 5	86 ± 4	$P = 0.04$		
Obese + GLP-1	86 ± 5	87 ± 5	100±6			
Mean Aortic Pressure (mmHg)						
Lean	94 ± 2	100±2	106±2	$P = 0.61$	$P = 0.01$	$P = 0.85$
Lean + GLP-1	99 ± 7	99 ± 6	105 ± 5			
Obese	108 ± 7	102±4	110±4	$P = 0.38$		
Obese + GLP-1	100±6	106 ± 7	122 ± 7			
Heart Rate (beats/min)						
Lean	147 ± 11	188 ± 9	221 ±9	$P = 0.95$	$P = 0.02$	$P = 0.69$
Lean + GLP-1	148 ± 8	187 ± 10	220 ± 8			
Obese	134 ± 9	176 ± 13	192 ± 18	$P = 0.08$		
Obese + GLP-1	137 ± 12	182 ± 14	197 ± 16			
Myocardial O_2 Consumption (μO_2/min/g)						
Lean	119 ± 14	185 ± 22	218 ± 18	$P = 0.45$	$P = 0.46$	$P = 0.53$
Lean + GLP-1	103 ± 14	172 ± 22	190 ±25			
Obese	113 ± 8	174 ± 25	184 ±27	$P = 0.63$		
Obese + GLP-1	110 ± 18	155 ± 21	196 ±28			
Myocardial Blood Flow (ml/min/g)						
Lean	1.03 ± 0.12	1.51 ±0.16	1.63 ± 0.12	$P = 0.07$	$P = 0.01$	$P = 0.43$
Lean + GLP-1	1.27 ± 0.16	1.59 ±0.20	1.81 ±0.22			

	Rest	Level 1	Level 2	GLP-1 Effect	Obesity	Interaction
Obese	0.91 ±0.12	1.32 ±0.22	1.42 ±0.21	$P = 0.99$		
Obese + GLP-1	0.96 ± 0.13	1.23 ± 0.18	1.47 ±0.24			
Myocardial Glucose Uptake (μmol/min/g)						
Lean	0.4 ± 0.2	0.3 ±0.2	0.8 ±0.2	$P = 0.11$	$P < 0.001$	$P = 0.37$
Lean + GLP-1	0.2 ± 0.2	1.2 ± 0.2	1.0 ± 0.2			
Obese	0.5±2	0.2 ±0.2	0.4 ±0.2	$P = 0.03$		
Obese + GLP-1	0.1 ±0.2	0.1 ±0.2	0.2 ±0.2			
Myocardial Glucose Extraction (%)						
Lean	7 ± 3	5±3	11 ±3	$P = 0.22$	$P = 0.02$	P=0.18
Lean + GLP-1	3 ± 3	19 ± 3	10 ± 3			
Obese	14 ± 3	4±3	5±3	$P = 0.06$		
Obese + GLP-1	2 ± 3	1 ±3	4±3			
Hemoglobin (g/dL)						
Lean	10.3 ± 0.6	10.7 ± 0.7	11.6 ± 1.1	$P = 0.02$	$P = 0.004$	$P = 0.83$
Lean + GLP-1	8.7 ± 0.6	9.3 ±0.7	9.6 ± 1.0			
Obese	11.9 ± 0.7	11.6 ± 0.8	12.2 ± 1.0	$P = 0.54$		
Obese + GLP-1	10.6 ± 0.9	11.6 ± 1.3	11.9 ± 1.1			
Coronary Venous PO_2 (mmHg)						
Lean	17.7 ± 0.9	17.1 ±0.9	16.2 ± 0.9	$P = 0.69$	$P < 0.001$	$P = 0.79$
Lean + GLP-1	18.3 ± 1.0	16.4 ±1.0	17.3 ± 1.1			
Obese	14.0 ± 1.2	12.2 ± 1.2	12.2 ± 1.2	$P = 0.82$		
Obese + GLP-1	14.5 ± 1.2	12.5 ± 1.2	12.1 ± 1.2			

Statistics are presented evaluating the effect of GLP-1 to modify exercise response within groups (GLP-1 effect), the effect of obesity on the pooled exercise responses (Obesity effect), and the interaction of GLP-1 effect and obesity effect on the exercise responses. Values are mean ± SE for lean (n = 7) and obese (n = 5) swine.