IMPAIRED CARDIOVASCULAR RESPONSES TO GLUCAGON-LIKE PEPTIDE 1 IN METABOLIC SYNDROME AND TYPE 2 DIABETES MELLITUS

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ABSTRACT

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IMPAIRED CARDIOVASCULAR RESPONSES TO GLUCAGON-LIKE PEPTIDE 1 IN METABOLIC SYNDROME AND TYPE 2 DIABETES MELLITUS

Recent advancements in the management of systemic glucose regulation in obesity/T2DM include drug therapies designed to utilize components of the incretin system specifically related to glucagon-like peptide 1 (GLP-1). More recently, GLP-1 has been investigated for potential cardioprotective effects. Several investigations have revealed that acute/sub-acute intravenous administration of GLP-1 significantly reduces myocardial infarct size following ischemia/reperfusion injury and improves cardiac contractile function in the settings of coronary artery disease, myocardial ischemia/reperfusion injury, and heart failure. Despite an abundance of data indicating that intravenous infusion of GLP-1 is cardioprotective, information has been lacking on the cardiac effects of iv GLP-1 in the MetS or T2DM population. Some important questions this study aimed to address are 1) what are the direct, dose-dependent cardiac effects of GLP-1 in-vivo 2) are the cardiac effects influenced by cardiac demand (MVO_2) and/or ischemia, 3) does GLP-1 effect myocardial blood flow, glucose uptake or total oxidative metabolism in human subjects, and 4) are the cardiac effects of GLP-1 treatment impaired in the settings of obesity/MetS and T2DM. Initial studies conducted in canines demonstrated that GLP-1 had no direct effect on

coronary blood flow *in-vivo* or vasomotor tone *in-vitro*, but preferentially increased myocardial glucose uptake in ischemic myocardium independent of effects on cardiac contractile function or coronary blood flow. Parallel translational studies conducted in the humans and Ossabaw swine demonstrate that iv GLP-1 significantly increases myocardial glucose uptake at rest and in response to increases in cardiac demand (MVO_2) in lean subjects, but not in the settings of obesity/MetS and T2DM. Further investigation in isolated cardiac tissue from lean and obese/MetS swine indicate that this impairment in GLP-1 responsiveness is related to attenuated activation of p38-MAPK, independent of alterations in GLP-1 receptor expression or PKA-dependent signaling. Our results indicate that the affects of GLP-1 to reduce cardiac damage and increase left ventricular performance may be impaired by obesity/MetS and T2DM.

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Chapter 1

Diabetes Mellitus, Metabolic Syndrome and Cardiovascular Disease

An increasing prevalence of Type 2 Diabetes Mellitus (T2DM) is a major national and global health concern with several important implications. The impact of this disease not only burdens our societies with morbidity and mortality, but also carries a large demand for structural, social and economic support. It is estimated that close to 350 million people worldwide have Diabetes Mellitus (1), and that over 8.3% of the United States population has this disease (2). T2DM typically accounts for approximately 90-95% of these cases (2). Once considered largely endemic to Western societies, T2DM has become an epidemic for many regional and cultural sectors worldwide. Amplifying the complications of the T2DM epidemic is the fact that these people are more likely to suffer from cardiovascular disease. Ultimately, as many as 90% of those with T2DM will suffer from cardiovascular disease in their lifetime (3).

Other major health concerns such as hypertension, dyslipidemia and obesity are often associated with hyperglycemia or overt T2DM (4, 5). Clusters of these conditions have been termed metabolic syndrome (MetS), and patients with MetS and/or T2DM represent a population with a significantly elevated burden of cardiovascular disease (**Figure 1-1**), the leading cause of death in the United States and globally (3, 4, 6-10). It is estimated that the total direct and indirect cost of coronary heart disease and heart failure exceeded ~140 billion dollars in the United States in 2010 (2).

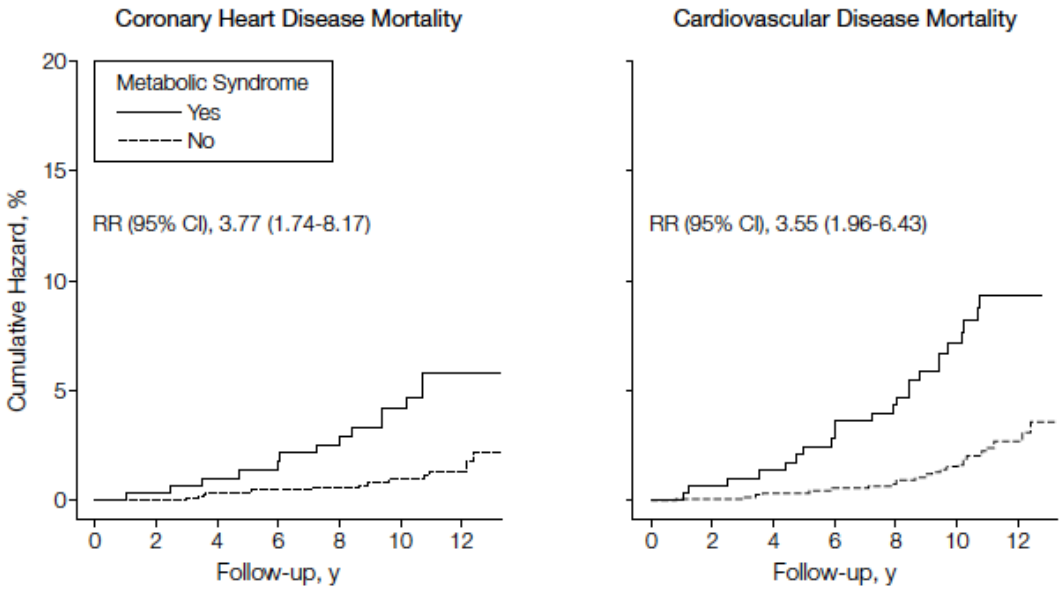

Figure 1-1 Coronary heart disease and total cardiovascular disease mortality risk associated with MetS. The year-to-year % incidence of mortality is depicted for a group of 1209 Finnish men age 42-60 y that were initially without cardiovascular disease, diabetes or cancer. RR – relative risk; CI – confidence interval; y – years. Modified from Lakka HM et al, JAMA, 2002 (10).

The disabling effects of heart disease are most evident during activity. Any increase in activity requires increased cardiac work to supply the body with oxygen rich blood and vital nutrition. Likewise, the increased demand on the heart must be matched with increased coronary blood flow. When the demands of cardiac work exceed perfusion the patient suffers cardiac ischemia, which often presents as angina pectoris. Thus, the relationship between coronary blood flow and metabolism (oxygen supply/demand) is being intensely investigated and therapies that act to rebalance this relationship are primary goals in the treatment and management of heart disease (11-52). Major ways in which this may be

accomplished are to increase coronary blood flow and shift cardiac metabolism to the utilization of more efficient substrate.

Advancements in the treatment of MetS, T2DM, and heart disease singly and collectively are direly needed to reduce the individual and societal burdens. While reasons for increased adverse cardiac events and outcomes in the MetS/T2DM population are still active areas of investigation, impaired regulation of coronary vascular function and reduced cardiac glucose metabolism are believed to be important factors (11, 13, 14, 17-20, 22, 26, 27, 29, 33, 40, 43, 53-56). Just as systemic glucose metabolism is impaired in this population, cardiac glucose metabolism is also impaired, and treatments that increase cardiac glucose uptake are being actively investigated (52-57). Therefore, common underlying pathologies may explain these disease associations, and offer common targets for intervention. Furthermore, when developing new therapeutic interventions with cardiovascular implications it is important to determine the safety and efficacy in the MetS/T2DM population.

Glucagon-like Peptide 1 and Systemic Glucose Regulation

Recent advancements in the treatment of T2DM include drug therapies designed to utilize components of the incretin system specifically related to glucagon-like peptide 1 (GLP-1). GLP-1 is an incretin hormone released from L-cells of the small intestine in response to feeding as a 7-36 peptide, i.e. GLP-1 (7-36). This peptide hormone is an agonist of the GLP-1 receptor (GLP-1R), a G-protein coupled receptor. In pancreatic beta cells this ligand/receptor interaction increases PKA activity and insulin secretion in a glycemia-dependent manner

(58, 59). GLP-1 (7-36) is also known to increase insulin sensitivity and reduce glucagon secretion, which amplifies the glucose lowering effect (60-67). Importantly, the insulinotropic effects of GLP-1 (7-36) are dependent on hyperglycemia, thus pharmacologic stimulation of this incretin pathway typically does not result in frank hypoglycemia (**Figure 1-2**).

Endogenously produced GLP-1 (7-36) has a very short plasma half-life of approximately 2 minutes (68, 69). It is cleaved by dipeptidyl-peptidase 4 (DPP-4) into GLP-1 (9-36), which is inactive as an insulinotropic/glucagonostatic agent and does not stimulate GLP-1R (70-74). A major advancement in the application of GLP-1 based therapies for systemic glucose control resulted from the discovery and subsequent research of a GLP-1R agonist, exendin-4. Exendin-4 is a peptide initially found in saliva of the Gila monster (*Heloderma suspectum*), a venomous lizard endemic to deserts in the southwestern United States and northwestern Mexico (75, 76). In addition to being a potent GLP-1R agonist, exendin-4 is resistant to the actions of DPP-4, and has an extended plasma half-life of ~25 minutes (**Figure 1-2**) (75, 77, 78).

A synthetic version of exendin-4, exenatide, was developed with a goal of systemic glucose management in the setting of T2DM, and in 2005 exenatide was the first GLP-1 based drug to be approved by the Food and Drug Administration (FDA) (79). Current FDA approved drugs based on GLP-1 include GLP-1R agonists with extended plasma half-life's (e.g. exenatide and liraglutide) and DPP-4 inhibitors (e.g. sitagliptin and vildagliptin) (80). Recent investigations suggest that therapies targeting GLP-1 pathways have beneficial pleotropic

effects including weight loss and improved lipid profiles, as well as more acute protective effect in the settings of stroke, heart failure and cardiac ischemia/reperfusion (62, 81-94). Therefore, additional investigations into the pleotropic effects of GLP-1 based therapies could be beneficial in not only further improving metabolic profiles in patients with MetS and/or T2DM, but also for acutely reducing morbidity and mortality in the settings of cardiac injury and failure. However, there is a paucity of information regarding the acute cardiac effects of GLP-1 in patients with MetS and/or T2DM.

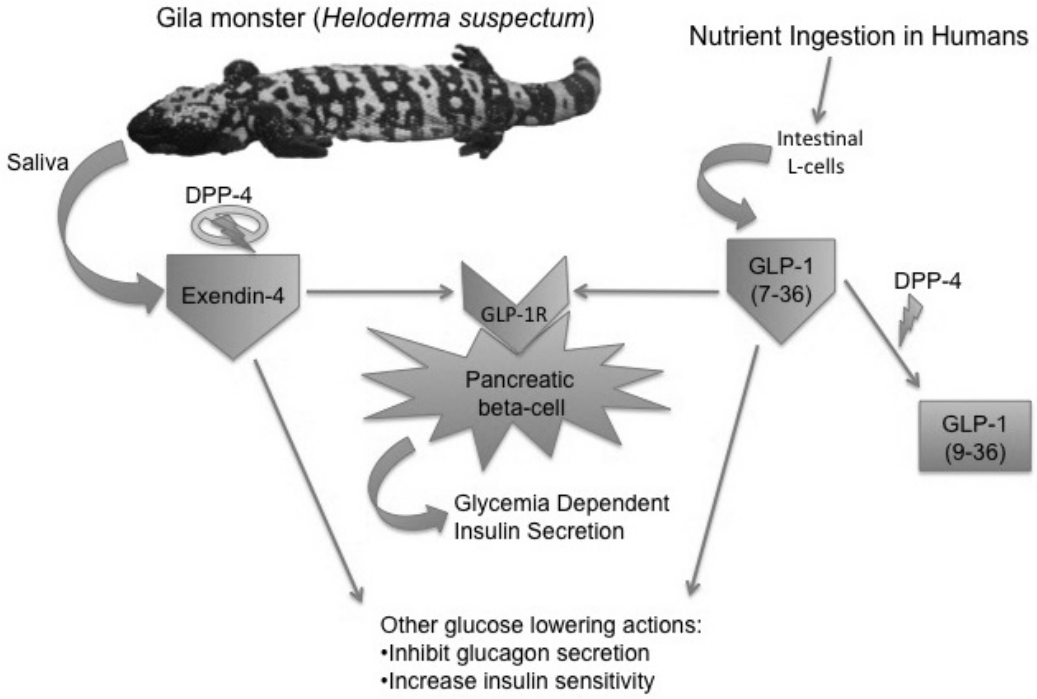

Figure 1-2 ***Depiction of the classical endocrine actions of GLP-1 (7-36) and the GLP-1R agonist Exendin-4 to regulate blood glucose.*** Notice that Exendin-4 is resistant to DPP-4 (Dipeptidyl peptidase-4), thus extending the plasma half-life.

Although GLP-1 based therapies are most commonly prescribed in the setting of MetS/T2DM, there is some evidence for diminished responsiveness in

this group. In comparison to lean healthy controls there is a reduced, yet still effective, glucose stimulated insulinotropic effect of GLP-1 (7-36) in the setting of obesity/T2DM. Specifically, low dose intravenous infusion (0.5 pmol/kg/min) of GLP-1 (7-36) was demonstrated to increase glucose stimulated insulin secretion rate in obese/T2DM patients to match that of untreated healthy controls, however when the control group was given the same glucose/GLP-1 treatment there was a greater response (95). While this diminished effect on pancreatic beta cell function did not hinder the application of GLP-1 for the purpose of enhancing insulin secretion, the potential for clinically significant reductions in the acute cardiac effects of GLP-1 in this population remain largely unknown. Thus, such differential responsiveness to the cardiac effects of GLP-1 warrants further investigation.

Glucagon-like Peptide 1 and the Heart

Recently, the cardiac effects of GLP-1 have come into focus. Clinical investigations have revealed that intravenous (iv) GLP-1 increases cardiac performance in the settings of heart failure, coronary artery disease and ischemia/reperfusion injury (87, 90, 96, 97). For example, patients with chronic heart failure had significantly improved left ventricular ejection fraction (LVEF), VO_2 max and 6 minute walk distance after receiving a 5 wk infusion of GLP-1 (7-36) (2.5 pmol/kg/min iv) (87). Other investigations have demonstrated that shorter term infusions of GLP-1 (7-36) at concentrations of 1.2 to 1.5 pmol/kg/min can improve LVEF and regional contraction following ischemia/reperfusion in patients with congestive heart failure, increase left ventricular function during

dobutamine stress tests in patients with coronary artery disease while decreasing post-test myocardial stunning, and reduce the need for insulin and inotropic support following coronary artery bypass grafting (94, 96, 97).

Information on the acute cardiac effects of GLP-1 in undamaged/healthy hearts is limited to a few studies in isolated rat and mouse hearts undergoing active coronary perfusion with physiologic buffer in a Langendorff preparation. In these studies, intracoronary administration of GLP-1 (7-36) increased coronary blood flow and myocardial glucose uptake in both rat and mouse heart (91, 93). However, while GLP-1 (7-36) increased left ventricular developed pressure (LVDP) in isolated mouse hearts, it decreased LVDP in isolated rat hearts. Under conditions of normal perfusion, GLP-1 (9-36) has only been tested in isolated mouse heart where it had no effect on LVDP or myocardial glucose uptake, but significantly increased coronary blood flow (93).

More extensive studies have been undertaken to examine the cardiac effects of GLP-1 based therapies under the condition of ischemia/reperfusion. A small clinical study revealed that continuous iv infusion of GLP-1 (7-36) started at the time of reperfusion in patients with congestive heart failure resulted in a ~30% increase in left ventricular ejection fraction (LVEF) after 72 hours compared to saline control (90). While it is not clear if this effect resulted from decreased ischemic damage, it is consistent with a multitude of animal studies which have demonstrated that either iv and/or intracoronary GLP-1 (7-36), GLP-1 (9-36), and GLP-1R agonists all reduce infarct size and increase left ventricular function following an ischemic event (91-93, 98-103). The DPP-4 inhibitor

sitagliptin has also been demonstrated reduce infarct size and increases left ventricular function in the setting of ischemia/reperfusion, as well as increase stroke volume in swine with heart failure (104-106).

A recent investigation in swine determined that exenatide improves several measures of LV function and reduces infarct size when administered via combined iv/subcutanious (SQ) routes 5 minutes prior to release of a 75 minute complete left circumflex occlusion and continued via SQ administration for two days (**Figure 1-3**) (107). Consistent with this investigation in swine, a very recent clinical trial demonstrated the same magnitude of infarct size reduction when iv exenatide was administered just prior to and following reperfusion (108). However, the GLP-1R agonist liraglutide had a neutral effect on cardiac function and infarct size when administered SQ for three days preceding a 40 minute complete occlusion of the left anterior descending coronary artery (LAD) (109). In the liraglutide study, reperfusion only persisted for 2.5 hours. It is not clear if the differences in effect between exenatide and liraglutide in these studies are due to the route/timing of administration, duration of reperfusion, or perhaps differential cardiac actions of these two GLP-1R agonists.

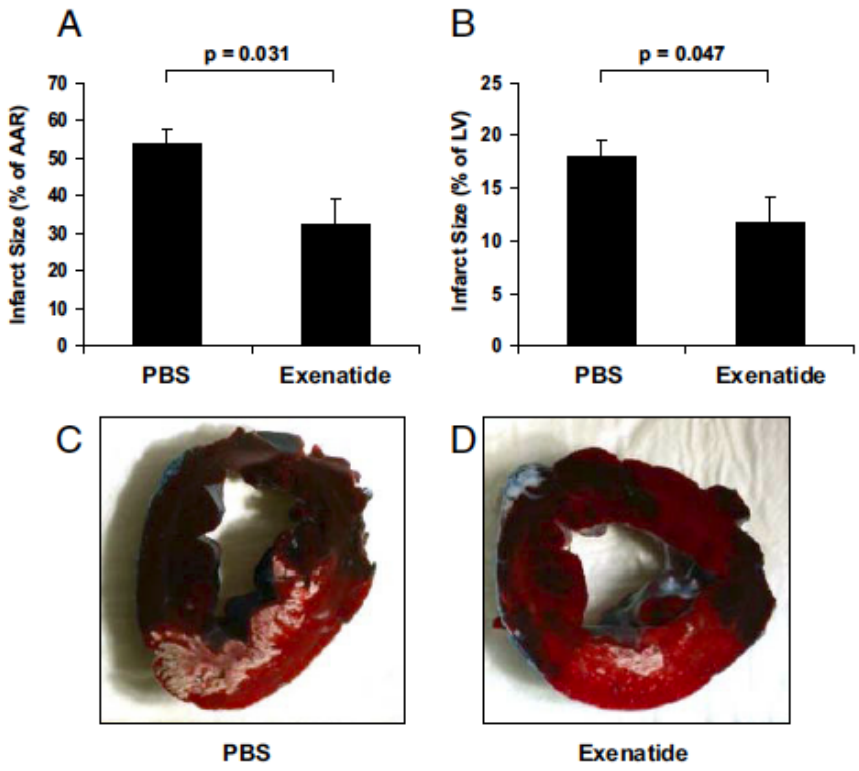

Figure 1-3 ***Exenatide reduces myocardial infarct size in swine after a 75-minute complete circumflex coronary artery occlusion.*** Myocardial infarct size as a percentage of the area at risk (AAR) **(A)**. As a percentage of the total left ventricle (LV) **(B)**. Phosphate-buffered saline (PBS) *n* = 9; exenatide n = 9. Representative images after Evans Blue and triphenyltetrazolium chloride staining are shown in **C and D**. **Blue** represents non-threatened myocardium, **red** indicates noninfarcted area within the area at risk, and **white** represents myocardial infarction. Figure taken from Timmers et al, 2009 (107).

The increased cardiac performance observed in patients with heart failure receiving iv GLP-1 (7-36) is representative of what has been observed in canines with cardiac-pacing induced heart failure. Investigations in canines have demonstrated that intravenous GLP-1 (7-36) improves cardiac function and increase myocardial glucose uptake in the setting of heart failure. At doses of 1.5 to 2.5 pmol/kg/min, intravenous GLP-1 (7-36) has been demonstrated to improve several parameters of cardiac function in canines with heart failure, such as LVEF, cardiac output and stroke volume (**Figure 1-4**) (88, 89, 94). Interestingly,

in this canine model of heart failure, GLP-1 (9-36) conveys nearly identical improvements in LV function, and increases in myocardial glucose uptake, as a dose/duration equivalent infusion of GLP-1 (7-36) (110).

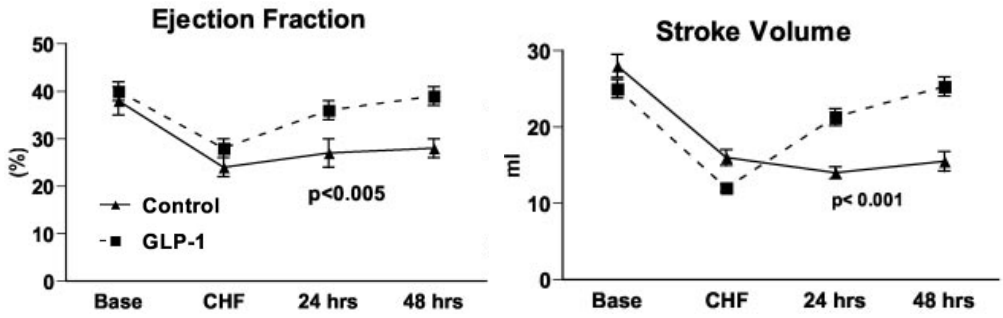

Figure 1-4 ***GLP-1 (7-36) significantly improves cardiac left ventricular function in canines with heart failure (n=16).*** Dose – 1.5 pmol/kg/min for 48 hours; CHF – Congestive Heart Failure. Modified from Nikolaidis LA et at, 2004 (89).

Glucagon-like peptide 1: Mechanisms of Cardiac Action

Myocardial expression of GLP-1R has previously been confirmed in canine heart, as well as the myocardium, coronary smooth muscle and coronary endothelium of mice (93, 94). Investigations using GLP-1R KO mice have been conducted to determine the role of GLP-1R in mediating the left ventricular performance enhancing effects of exendin-4, GLP-1 (7-36), and GLP-1 (9-36). The results of these investigations are inconsistent, and clear explanations are lacking. Exendin-4, GLP-1 (7-36), and GLP-1 (9-36) all increased cardiac performance following ischemia/reperfusion in isolated hearts from both wild type and GLP-1R KO mice (93, 102). Although GLP-1R dependent pathways may also contribute to the beneficial cardiac effects of exendin-4, these findings suggest that non-GLP-1R pathways mediated the direct cardiac actions in this

study. It was also determined that the use of a DPP-4 inhibitor abolished the cardiac actions of GLP-1 (7-36) in GLP-1R KO, but not wild type, mouse hearts (93). Thus indicating that in the absence of a cardiac GLP-1R, the direct cardiac actions of GLP-1 are mediated by GLP-1 (9-36), but that the effects of intact GLP-1 (7-36) are GLP-1R dependent. Such similar effects of GLP-1R agonists, GLP-1 (7-36) and GLP-1 (9-36) have also been observed in large animal models, as discussed above (107, 110). Taken together, these data indicate that exendin-4 and GLP-1 (9-36) have cardioprotective value not related to the cardiac GLP-1R, but that the direct cardiac actions of intact GLP-1 (7-36) are GLP-1R dependent. Importantly, GLP-1R agonists, GLP-1 (7-36), and GLP-1 (9-36) have all been demonstrated to convey beneficial cardiac effects. Furthermore, continued systemic administration of GLP-1 (7-36) also increases circulating GLP-1 (9-36) thus taking advantage of the cardioprotective potential of these two separate, yet related, peptides (72, 111).

Potential physiological mechanisms for the actions of GLP-1 based therapies to increase left ventricular function and reduce infarct size include 1) inotropic effects 2) effects on substrate metabolism, and 3) effects on coronary blood flow. It is conceivable that the increases in cardiac function observed with treatments based on GLP-1 are due to inotropic actions, and not necessarily related to cardioprotection. However, there is evidence that inotropic and/or adrenergic stimulation are not responsible for increased cardiac performance in the setting of GLP-1 based therapy.

GLP-1 as an inotrope

Classic cardiac inotropic/adrenergic stimulation is mediated in part by an increase in cAMP production and subsequent activation of PKA. While GLP-1R activation in pancreatic beta cells signals through this pathway, several investigations suggest that the cardiac actions of GLP-1 are not mediated by cAMP/PKA (91, 94), although not a point of complete agreement (93, 101). Furthermore, since these therapies are effective in isolated hearts and are not associated with increased heart rate it is not likely that sympathetic/adrenergic activation is involved (89, 91). Finally, previous studies have demonstrated a lag time of hours between the increases in glucose uptake and the cardiac performance-enhancing effects, which is distinctly different than classic sympathetic/adrenergic stimuli (94). The majority of evidence suggests that some combination of augmented myocardial glucose uptake, greater mechanical efficiency, increased coronary blood flow, and/or myocardial tissue preservation is responsible for the gains in left ventricular performance (89, 93, 102).

GLP-1 and Myocardial Glucose Uptake

Increasing myocardial glucose uptake has been a target for cardioprotection for decades (52, 57, 112-117). Some of the theory behind this approach is based on knowledge that glucose has a high P/O ratio (ATP produced per oxygen consumed), and that increased glucose metabolism can drive a reduction in fatty acid metabolism (i.e. Randle Cycle) (112, 118-120). At the level of mitochondrial oxidative metabolism, fatty acid has a lower P/O ratio, greater futile cycling, and generates more reactive oxygen species than does glucose metabolism (53, 118). Thus, increasing myocardial glucose uptake

should improve cardiac efficiency, allow maintenance of a high-energy state, protect cardiac tissue from oxidative damage and increase performance in underperfused tissue. This would be beneficial not only in terms of protecting the heart from ischemic injury, but also in terms of maintaining cardiac output to sustain tissue function and survival at an organismal level.

Experimental data support the rationale behind targeting myocardial substrate metabolism for the purpose of disease intervention. Earlier studies from our lab have demonstrated that insulin increases cardiac function and efficiency in the setting of ischemia, effects also related to increased myocardial glucose uptake (52, 57). While this was an important finding, the clinical perspectives for using insulin to mitigate myocardial injury in an acute setting have been limited. Reasons for this limitation include problems associated with the acute use of intensive insulin therapy in patients with failing hearts, such as a significant risk of hypoglycemia and hypokalenmia, as well as the requirement for large volumes of iv glucose solution to maintain euglycemia (121-123). High iv fluid requirements result in higher volume loads on the heart (i.e. increased metabolic demand), likely offsetting the gains in metabolic efficiency acquired by increased myocardial glucose utilization.

GLP-1 has been demonstrated to increase myocardial glucose uptake and cardiac efficiency (**Figure 1-5**), with significantly less risk and lower volume requirements than insulin (89). Previous studies in isolated hearts from mice and rats indicate that intracoronary GLP-1 (7-36) increases myocardial glucose uptake under control conditions and following ischemia (91, 93). Other studies in

canines have determined that systemic administration of both GLP-1 (7-36) and GLP-1 (9-36) increase myocardial glucose uptake in the setting of pacing induced heart failure, an effect demonstrated to work both independently and synergistically with insulin (89, 110).

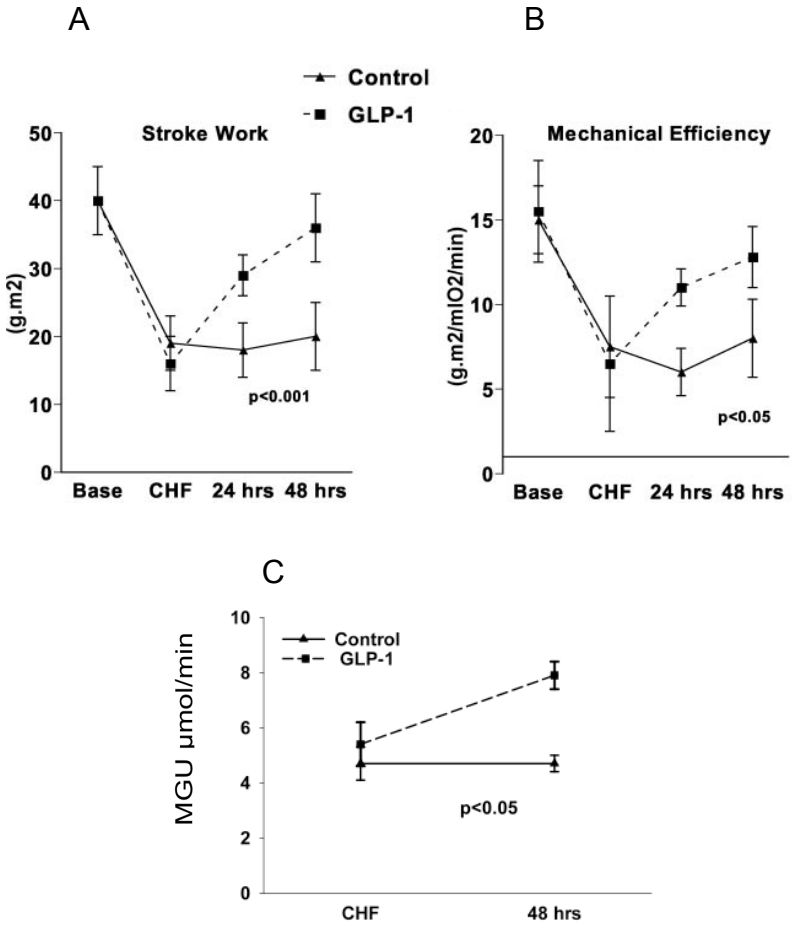

Figure 1-5 ***GLP-1 (7-36) significantly increases cardiac stroke work (A), mechanical efficiency (B), and glucose uptake (C) in canines with heart failure*** **(*n = 16*).** Dose – 1.5 pmol/kg/min for 48 hours; CHF – Congestive Heart Failure. Modified from Nikolaidis LA et at, 2004 (89).

Both GLP-1 (7-36) and GLP-1 (9-36) have been demonstrated to increase left ventricular performance and myocardial glucose uptake (93, 102, 110).

Which suggest that the cardiac insulinomimetic effects are GLP-1R independent. There are recent data indicating that this effect is mediated by non-canonical GLP-1 signaling involving NO, p38-MAPK, and GLUT-1. Inhibition of p38-MAPK with SB203580 or inhibition of nitric-oxide synthase (NOS) with nitro-L-arginine (N-LA) was able to reduce the salutary effects of systemic GLP-1 (7-36) on myocardial glucose uptake in canines (94). Likewise, it has been demonstrated that GLP-1 (7-36) increases myocardial glucose uptake, p38-MAPK activity, nitric oxide production, and membrane GLUT-1 expression when administered via intracoronary infusion into isolated rat hearts or when administered intravenously in canines (91, 94).

Acute increases in striated muscle (i.e. skeletal and cardiac) glucose uptake are classically attributed to membrane insertion of cytoplasmic GLUT-4 vesicles (124-126). GLUT-1 is typically thought of as managing basal glucose uptake, not acute changes (126). However, cardiac GLUT-1 has become increasingly recognized for a role in mediating acute changes in glucose uptake (127, 128). GLP-1 (7-36) has been demonstrated to significantly increase myocardial glucose uptake in normally perfused isolated rat hearts within minutes of administration without effecting membrane GLUT-4, but increasing membrane GLUT-1 content (91). This acute time frame indicates that these effects are not mediated by transcriptional regulation, but rather membrane trafficking. In the post-ischemic rat heart GLP-1 (7-36) acutely increased membrane content of both GLUT-1 and GLUT-4 (91). Thus, current data indicate that GLP-1 augments

myocardial glucose uptake via mechanisms involving NO, p38-MAPK, and GLUT-1 with conditional effects on GLUT-4.

GLP-1 stimulated increases in myocardial glucose uptake were associated with the same cellular signaling cascade when administered into the coronary circulation of isolated rat hearts, and administered intravenously in canines with heart failure. However, this insulinomimetic effect occurred within 30 minutes in isolated rat heart (91), while a longer infusion time of approximately 6 hours was required in canines (94). This difference is most likely due to the fact that a longer infusion time was used with systemic administration to reach plasma concentrations equal to that used for intracoronary infusion (~400 to 500 pmol/L).

The salutary effects on myocardial glucose uptake may be particularly relevant to patients with MetS and/or T2DM. These conditions are associated with a shift in myocardial substrate metabolism, which decreases the contribution of glucose to total cardiac energy supply while increasing fatty acid uptake (53-56). Drug therapies based on GLP-1 may improve cardiac glucose metabolism in this patient population. In addition to enhancing the actions of insulin on myocardial glucose uptake, GLP-1 has been demonstrated to increase cardiac glucose uptake independently of insulin, and by mechanisms not associated with insulin mediated glucose uptake. This is promising since insulin resistance is common among these patients. However, ***the cardiometabolic effects of GLP-1 have not been directly investigated in the settings of obesity/MetS or T2DM***. While it is speculated that GLP-1 may be of particular benefit patients with

MetS/T2DM, it remains possible that the cardiac effects are impaired in this patient population.

GLP-1 and Coronary Blood Flow

There are several lines of evidence that GLP-1 may protect the heart from ischemic injury by augmenting coronary blood flow. Intracoronary GLP-1 (7-36) has been shown to increase coronary blood flow in isolated mouse and rat hearts (91, 93). Furthermore, intracoronary administration of both GLP-1 (7-36) and GLP-1 (9-36) have been demonstrated to increase coronary blood flow in isolated hearts from both wild type and GLP-1R KO mice under conditions of normal perfusion, and following ischemia (93). Other studies have demonstrated that GLP-1 (7-36) induces vasodilation in isolated aortic, femoral, and pulmonary arteries from rats (129-132).

Systemic administration of GLP-1 (7-36) into canines was demonstrated to modestly increase coronary blood flow in one study; however, this increase in blood flow was also associated with an increase in MVO_2. Thus it is unclear if the GLP-1 was acting as a coronary vasodilator, or if the increased coronary blood flow was a direct result of elevated metabolic demand (89). In other studies, neither iv GLP-1 (7-36) nor GLP-1 (9-36) increased coronary blood flow in canines (94, 110). There were also no increases in MVO_2 in these other canine studies. Acute infusion of GLP-1 (7-36) (10 pM/kg/min IV) into swine immediately following cardiac fibrillation/resuscitation had no effect on coronary blood flow at 1 or 4 hours, but did increase the flow response to intracoronary adenosine (an index of coronary flow reserve) at these time points (133).

Taken together, these data indicate that GLP-1 may have direct cardiac effects to increase coronary blood flow. However, the effects of intracoronary GLP-1 vs. that of iv GLP-1 have not been tested in the same animal species. Therefore, it is unknown if the differential responses are due to the route of administration and/or species differences. Furthermore, the effects of GLP-1 on coronary blood flow and vascular tone are unknown in humans and in the settings of MetS/T2DM. Further studies to determine the effects of GLP-1 on coronary blood flow and vascular tone are needed.

Summary

Patients with MetS and/or T2DM have an elevated risk for cardiovascular disease, the leading cause of mortality in the United States and globally (3, 4, 8-10). While the reasons for increased adverse cardiac events and outcomes in the MetS and T2DM populations are still active areas of investigation, coronary microvascular dysfunction and reduced cardiac glucose metabolism are implicated in having key roles (14, 26, 42, 53-55). Thus, improving myocardial oxygen delivery and promoting more efficient cardiac substrate utilization; i.e. restoring the balance of myocardial oxygen supply and demand, are primary targets for advancing the treatment and management of obesity-related cardiovascular disease.

Therapies based on GLP-1 are currently used for systemic glucose management in the setting of T2DM, and recently there has been interest in potential cardioprotective actions of GLP-1. Multiple studies have revealed that GLP-1 based drugs increase left ventricular performance in the damaged/failing

hearts of humans, swine, canines, rabbits, rats and mice (**Table 1-1**). Increases in myocardial glucose uptake and/or coronary blood flow are associated with this cardiac performance-enhancing effect, and thought to play a critical role (**Table 1-1**). These findings indicate that GLP-1 may be beneficial in treating the underlying pathology of obesity related heart disease. While the cellular mechanisms responsible for mediating the cardiac actions of GLP-1 are still under investigation, p38-MAPK and/or PKA dependent signaling pathways have been implicated (93, 94).

Table 1-1 *Cardiac effects of Glucagon-like peptide 1, GLP-1R agonists, and DPP-4 inhibition*

Subject	Condition	LV Function and infarct size	MGU	CBF	Citation
GLP-1 (7-36)	Normal perfusion, ex-vivo	• LVDP (↓↓) rat, (↑↑) mouse • dP/dt max (↓↓) rat	(↑↑↑) rat, and (↑↑↑) mouse	(↑↑↑) rat, and increased mouse	91, 93
	Ischemia – Reperfusion	• LVEF (↑↑) human, (↑↑) rabbit • LVDP (↑↑) rat, (↑↑↑) mouse • dP/dt max (↑↑↑) rat • LVEDP (↓↓↓) rat • IS (↓↓) rabbit, NS rat	(↑↑↑) rat	NS in rat, increased in mouse	90, 98, 91, 93, 92
	Heart failure	• LVEF (↑↑) human, (↑↑↑) and (↑↑) canine • CO (↑↑) canine • SV (↑↑↑) canine • dP/dt max (↑↑↑) canine • LVEDP (↓↓↓) canine	(↑↑↑), (↑↑) and (↑↑↑) canine	NS, (↑↑), and NS canine	87, 89, 110, 94
GLP-1 (9-36)	Normal perfusion, ex-vivo	• NS mouse	NS in mouse	Increased in mouse	93
	Ischemia – Reperfusion	• LVDP (↓↓) rat • dP/dt max (↓↓) rat • LVDP (↑↑) and (↑↑↑) mouse • IS NS rat, (↓↓) mouse	NA	Increased in mouse	99, 93, 102,
	Heart Failure	• CO (↑↑) canine • dP/dt (↑↑↑) canine • LVEDP (↓↓↓) canine	(↑↑↑) canine	NS	110
Exendin-4 (GLP-1R agonist)	Normal perfusion, exv-vivo	• LVDP NS mouse	NA	NA	93
	Ischemia – Reperfusion	• LVDP (↑↑↑), and (↑↑↑) mouse • IS (↓↓↓) mouse	NA	NA	93, 102
Exenatide (GLP-1R agonist)	Ischemia – Reperfusion	• LVEF (↑↑) swine • dP/dt max (↑↑) swine • IS (↓↓) swine	NA	NA	107
Sitagliptin (DPP-4 inhibitor)	Normal perfusion	• LVDP NS in mouse	NA	NA	105
	Ischemia – Reperfusion	• LVDP (↑↑) mouse • IS (↓↓) mouse	NA	NA	105, 104
	Heart Failure	• SV (↑↑) swine	NA	NA	106

Figure Legend: LVDP – Left Ventricular Developed Pressure, dP/dt max – Maximum rate of left ventricular pressure development, LVEF – Left Ventricular Ejection Fraction, LVEDP – Left Ventricular End Diastolic Pressure, CO – Cardiac Output, SV – Stroke Volume, IS – Infarct Size, NS – Not Significant, N/A – Not Available, (↑↑) ≥ 20% increase, (↑↑↑) ≥ 50% increase, (↓↓) ≥ 20% decrease, (↓↓↓) ≥ 50% decrease

Specific Aims

Despite an abundance of data indicating that intravenous infusion of GLP-1 is cardioprotective (**Table 1**), information is lacking on the acute/sub-acute cardiac effects of GLP-1 in the MetS or T2DM population. It was recently recognized that the insulinotropic effect of GLP-1 is diminished, although still effective, in the setting of obesity/T2DM (95). However, whether obesity, MetS, and/or T2DM alter the cardiac actions of GLP-1 has not been directly evaluated. Thus, while GLP-1 may be useful in the treatment and management of obesity related heart disease by improving coronary microvascular function and myocardial glucose uptake, there is a distinct possibility that the cardiac actions of GLP-1 are impaired in the settings of obesity/MetS and T2DM. Some important questions remaining are 1) what are the direct, dose-dependent cardiac effects of GLP-1 *in-vivo* 2) are the cardiac effects influenced by cardiac demand (MVO_2) and/or ischemia, 3) does GLP-1 effect myocardial blood flow, glucose uptake or total oxidative metabolism in human subjects, and 4) are the cardiac effects of GLP-1 treatment impaired in the settings of obesity/MetS and T2DM.

Accordingly, the goal of the present application is to more fully elucidate the cardiac actions of GLP-1, determine if these actions are impaired in the setting of obesity/MetS, and uncover potential mechanisms of impairment. We hypothesize that GLP-1 will have direct cardiac actions which increase myocardial blood flow, glucose uptake, and function during ischemia. We also hypothesize that the milieu of obesity, MetS and T2DM may impair the cardiac

effects of GLP-1 by mechanisms involving GLP-1R expression, p38-MAPK signaling and/or PKA signaling. This hypothesis will be examined by translational studies in open-chest canines, our novel Ossabaw swine model of obesity/MetS, and humans with MetS/T2DM. We propose to accomplish our goal by pursuing the following Specific Aims:

Aim 1: ***Determine the acute, dose-dependent cardiac effects of intracoronary GLP-1 under conditions of normal coronary perfusion and during ischemia.*** Approach: Open-chest anesthetized canines will be studied under conditions of normal coronary perfusion and during ischemia by measuring indices of cardiac function, as well as myocardial blood flow, glucose uptake, and total oxidative metabolism with and without acute intracoronary infusion of GLP-1 (7-36) (10 pmol/L to 1 nmol/L). Myocardial biopsies will be obtained for determination of GLP-1 receptor localization.

Aim 2: ***Test the hypothesis that obesity/MetS impairs the cardiac responses to GLP-1, and investigate potential mechanisms of such impairment.*** Approach: Parallel studies will be conducted in humans and swine. Human subjects with MetS/T2DM will be studied using a triple-tracer PET approach to measure myocardial blood flow, glucose uptake, fatty acid uptake, and total oxidative metabolism with and without GLP-1 (7-36) (1.5 pmol/kg/min iv). Measurements of cardiac function will be made using impedance cardiography. Control data will be available from identical studies in lean subjects participating in a separate investigation. Concurrently, chronically instrumented lean and MetS Ossabaw swine will be studied at rest and during graded treadmill exercise by

measuring hemodynamic variables, as well as myocardial blood flow, glucose uptake, and total oxidative metabolism with and without GLP-1 (7-36) (1.5 pmol/kg/min iv). Myocardial biopsies will be obtained from lean and MetS swine to examine potential signaling pathways activated by GLP-1.

This project has direct clinical significance, and will demonstrate translation between mechanistic animal studies and effects observed in humans. These studies will be informative regarding mechanisms of, and novel treatment modalities for, MetS-induced cardiovascular disease.

Chapter 2

Intracoronary glucagon-like peptide 1 preferentially augments glucose uptake in ischemic myocardium independent of changes in coronary flow

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Abstract

We examined the acute dose-dependent effects of intracoronary GLP-1 (7-36) on coronary vascular tone, cardiac contractile function and metabolism in normal and ischemic myocardium. Experiments were conducted in open chest, anesthetized dogs at coronary perfusion pressures (CPP) of 100 and 40 mmHg before and during intracoronary GLP-1 (7-36) infusion (10 pmol/L to 1 nmol/L). Isometric tension studies were also conducted in isolated coronary arteries. Cardiac and coronary expression of GLP-1 receptors (GLP-1R) was assessed by Western Blot and immunohistochemical analysis. GLP-1R was present in myocardium and the coronary vasculature. Tension of intact and endothelium-denuded coronary artery rings was unaffected by GLP-1. At normal perfusion pressure (100 mmHg), intracoronary GLP-1 (7-36) (targeting plasma concentration 10 pmol/L to 1 nmol/L) did not affect blood pressure, coronary blood flow, or myocardial oxygen consumption (MVO_2); however, there were modest reductions in cardiac output and stroke volume. In untreated control hearts, reducing CPP to 40 mmHg produced marked reductions in coronary blood flow (0.50 ± 0.10 to 0.17 ± 0.03 ml/min/g; $P < 0.001$) and MVO_2 (27 ± 2.3 to 15 ± 2.7 µl O_2/min/g; $P < 0.001$). At CPP = 40 mmHg, GLP-1 had no effect on coronary blood flow, MVO_2, or regional shortening, but dose-dependently increased myocardial glucose uptake from 0.11 ± 0.02 µmol/min/g at baseline to 0.17 ± 0.04 µmol/min/g at 1 nM GLP-1 ($P < 0.001$). These data indicate that acute, intracoronary administration of GLP-1 (7-36) preferentially augments glucose metabolism in ischemic myocardium, independent of effects on cardiac contractile function or coronary blood flow.

Keywords: coronary blood flow, myocardial oxygen consumption, GLP-1, canine, cardiac metabolism

Introduction

Glucagon-Like Peptide 1 (GLP-1) is an insulinotropic hormone released from intestinal L-cells in response to feeding. The full-length peptide GLP-1 (7-36) is a ligand for the G-protein coupled GLP-1 receptor (GLP-1R). GLP-1 (7-36) is quickly degraded by circulating dipeptidyl peptidase 4 (DPP-4) to yield GLP-1 (9-36), which does not activate the GLP-1R, and is inactive as an insulinotropic agent (134). Endogenous plasma concentrations of GLP-1 (7-36) observed in humans range from ~5 to 30 pmol/L in a fasting state to ~30 to 40 pmol/L following a mixed meal (135-138). Current GLP-1 based therapeutics for the treatment of type 2 diabetes mellitus include GLP-1R agonists with long circulating half-lives (e.g. exenatide), and DPP-4 inhibitors (e.g. sitagliptin) (139). Although these therapies have been linked with cardioprotective mechanisms, they are not currently prescribed for this purpose (53, 88-94, 100, 101, 110, 140).

Recent research on the cardiac effects of GLP-1 indicates that systemic infusion of the full-length (7-36) peptide influences cardiac contractile function and glucose utilization. Studies in humans and in canines demonstrate that intravenous administration of GLP-1 (7-36) (1.5 to 2.5 pmol/kg/min) for 24 to 72 hours improves cardiac contractile performance following coronary artery occlusion, (88, 90) and pacing-induced heart failure (89, 94, 110). This improvement in cardiac function is associated with an increase in myocardial glucose utilization and occurs both independently and synergistically with insulin

(89, 110). Other studies have documented that GLP-1 (7-36) induces vasodilation of isolated aortic, femoral, and pulmonary arteries in rats (129-132), and increases coronary blood flow in normal and post ischemic isolated murine hearts (91, 93). Systemic administration of recombinant GLP-1 (7-36) has also been shown to increase coronary blood flow *in-vivo* in canines with pacing induced dilated cardiomyopathy (89). However, cardiac contractile performance and myocardial oxygen consumption (MVO_2) were also increased by GLP-1 in this study. Thus, it is unclear if the changes in coronary flow were the result of direct actions of GLP-1 on the coronary circulation or if they were the result of increased metabolic demand, i.e. metabolic vasodilation. While it has been demonstrated by Western Blot that GLP-1R is present within canine myocardium (94), the distribution of GLP-1R within canine heart, including the possible presence within coronary microvessels, has not previously been determined. Taken together, these findings suggest that GLP-1 (7-36) could protect the heart from ischemic injury by shifting cardiac substrate metabolism toward glucose and/or by augmenting myocardial perfusion (52, 53). Whether these effects of GLP-1 *in-vivo* occur acutely (minutes vs. hours) and/or are dependent on whole-body responses to systemic exposure to GLP-1 has not been assessed.

This study tested the hypothesis that acute administration of GLP-1 (7-36) directly into the coronary circulation augments coronary blood flow, myocardial glucose metabolism and cardiac contractile function in normal and ischemic myocardium in a dose-dependent manner. Experiments were conducted in open chest anesthetized dogs at coronary perfusion pressures (CPP) of 100 and 40

mmHg before and during intracoronary GLP-1 (7-36) infusion (intracoronary concentrations of 10 pmol/L to 1 nmol/L). Coronary vascular effects of GLP-1 (7-36) (10 pmol/L to 1 nmol/L) were also assessed by isometric tension studies in isolated coronary arteries. In addition, cardiac and coronary expression of GLP-1R was determined by Western Blot analysis and immunohistochemistry with confocal microscopy.

Methods

Surgical preparation. Animal procedures used for this investigation were approved by the Institutional Animal Care and Use Committee and conducted in accordance with guidelines in the *Guide for the Care and Use of Laboratory Animals*. Adult male mongrel dogs weighing ~20 kg were sedated with morphine (3mg/kg, subcutaneous) and anesthetized with α-chloralose (100 mg/kg, intravenous). Following intubation, animals were ventilated with room air supplemented with oxygen, and positive end-expiratory pressure was held at ~2 cm H_2O to prevent atelectasis. Aortic pressure was measured through a catheter introduced into the thoracic aorta through the left femoral artery. Another catheter, inserted into the right femoral vein was used to maintain anesthesia, and administer sodium bicarbonate as needed to maintain pH within normal physiological limits. The right femoral artery was also catheterized to supply blood to a pump perfusing the left anterior descending coronary artery (LAD). A left lateral thoracotomy was then performed to expose the heart. The LAD was isolated distal to its first major diagonal branch, cannulated, and connected to the extracorporeal perfusion system. Coronary perfusion pressure was maintained

constant at 100 or 40 mmHg during the experimental protocol by a servo-controlled roller pump. Coronary blood flow was measured within the extracorporeal perfusion circuit with an in-line flow transducer (Transonic Systems, Inc., Ithaca, NY, USA). Intravenous heparin was administered (500 U/kg) to prevent coagulation. The great cardiac vein was also cannulated to collect blood for metabolic analysis of the LAD perfusion territory. Left ventricular (LV) pressure and cardiac output (CO) were measured with a Millar® Mikro-Tip SPR-524 catheter in the LV (Millar Instruments, Inc., Houston, TX, USA), and a flow probe around the root of the aorta (Transonic Systems, Inc) respectively. Regional contractile function was determined using ultrasonic crystals (Sonometrics, Inc., London, ON, Canada) placed in myocardium of the LAD perfusion territory at a depth of ~7mm. These data were analyzed by custom-made software developed in Matlab® (Mathworks®, Natick, MA, USA). Percent segment shortening was calculated as [(end diastolic length – end systolic length) / end diastolic length]. End diastolic length was taken at the beginning of the positive deflection of LV dP/dt (rate of pressure delevelopment), and end systolic length was taken 20 ms before the peak negative deflection of dP/dt, corresponding with the dicrotic notch of the aortic pressure recording, as previously reported (52, 141). All materials which were implanted into the animals or in contact with the circulation (i.e. cannulas, catheters and flow probes) were cleaned with the multi-enzyme detergent Enzyte™ (Decon Labs, Inc., King of Prussia, PA, USA) for the removal of blood, proteins, and other biological

material, then rinsed thoroughly with deionized water and allowed to dry prior to use.

Acute dose-dependent effects GLP-1 (7-36) on cardiovascular hemodynamics. Acute *in-vivo* experiments were conducted in open chest, anesthetized dogs (*n* = 9) at CPP of 100 and then 40 mmHg before and during intracoronary GLP-1 (7-36) infusion (10 pmol/L to 1 nmol/L). Following cannulation of the LAD, hemodynamic and contractile function parameters were allowed to stabilize for ~10-15 min before acquiring baseline data. After baseline parameters were measured and blood samples obtained, an infusion of GLP-1 (7-36) (G8147; Sigma Aldrich, St. Louis, MO, USA) was initiated into the LAD perfusion line at a constant infusion rate in order to obtain coronary plasma concentrations of 10 pmol/L to 1 nmol/L. Plasma flow was calculated as [(1-hematocrit) X coronary blood flow]. After completion of the GLP-1 (7-36) infusion at CPP = 100 mmHg, a 10 minute washout period was allowed before CPP was lowered to 40 mmHg. Following stabilization of hemodynamic parameters at CPP = 40 mmHg (~5 to 10 min), baseline data were acquired and the intracoronary infusion of GLP-1 (7-36) repeated. Data were averaged from 10 consecutive beats following ~5 min of GLP-1 (7-36) administration at each dose.

Metabolic Analysis. Arterial and coronary venous blood were collected simultaneously, immediately sealed, and placed on ice. The samples were analyzed for pH, PCO_2, PO_2, O_2 content, hematocrit, glucose concentration, and lactate concentration with an Instrumentation Laboratories automatic blood gas analyzer (GEM Premier 3000; Instrumentation Laboratory Company, Bedford,

MA, USA) and CO-oximeter (682) system (Instrumentation Laboratory Company). LAD perfusion territory was estimated to be 30% of total heart weight, as previously described by Feigl *et al.* (141) MVO_2 (µl O_2/min/g) was calculated as [coronary blood flow X (arterial O_2 content – coronary venous O_2 content)]. Fick principle was also used to calculate glucose and lactate uptake.

Functional assessment of isolated coronary artery rings. Isometric tension studies were conducted in both endothelium intact and denuded isolated coronary artery rings treated with the same concentrations of GLP-1 used in the *in-vivo* studies (10 pmol/L to 1 nmol/L). For these experiments canine hearts were excised, immediately rinsed, and bathed with ice-cold saline. Epicardial LV coronary arteries were dissected from the heart, cleaned of perivascular fat, and cut into ~3 mm rings. These fresh arterial rings were mounted in organ baths with warm oxygenated Krebs solution and brought to an optimal preload of ~4 g, as previously described (142). Rings were then pre-constricted with the thromboxane A_2 mimetic U46619 (1 µmol/L). Graded doses of GLP-1 (7-36) were then added to the baths in a cumulative manner. Presence or absence of functional endothelium was assessed by reactivity to acetylcholine (10 µmol/L). Viability of arterial smooth muscle was determined by reactivity to sodium nitroprusside (20 µmol/L).

Western blot analysis. Following excision of the hearts, transmural LV samples were quickly isolated, snap frozen in liquid N_2, and stored at -80˚C. These samples were later homogenized in lysis buffer, and protein was quantitated using a DC protein assay. 35 µg of protein was loaded onto a 7.5%

acrylamide gel and transferred overnight. Membranes were blocked prior to a 1 hour incubation with rabbit polyclonal GLP-1R antibody (ab39072; Abcam, Cambridge, MA, USA) in blocking buffer with 0.1% Tween 20 at ambient temperature. Membranes were washed and incubated for 1 hour with IRDye 800 donkey anti-rabbit secondary antibody. Immunoreactivity to GLP-1R was determined by a Li-Cor Odyssey system (Li-Cor Biosciences, Lincoln, NE, USA). Band size was determined using comparison with a standard protein ladder (161-0375; BIO Rad, Hercules, CA, USA).

Immunohistochemistry and confocal microscopy. Fresh LV cardiac samples were snap frozen in liquid N_2 and stored at -80˚C. Frozen LV samples were then embedded in Tissue-Tek® OCT™ (Sakura Finetek USA, inc, Torrance, CA, USA) and sliced into 10 µm sections. Slices were incubated in mouse monoclonal antibodies against cardiac troponin I (ab10231; 1:1,000), 4',6-diamidino-2-phenylindole (DAPI; 100 ng/ml), and rabbit polyclonal antibodies raised against GLP-1R (ab39072; 1:25) for one hour. 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 using an Olympus2 single photon confocal microscope (Olympus America Inc., Center Valley, PA, USA).

Statistical Analyses. Data are presented as mean ± SE. Statistical comparisons were made with one-way repeated measures analysis of variance (ANOVA). For all comparisons, $P < 0.05$ was considered statistically significant. When significance was found with ANOVA, a Student-Newman-Keuls multiple

comparison test was performed to identify differences between treatment levels and/or CPP.

Results

Cardiac and coronary expression of GLP-1R. Western Blot analysis demonstrated staining of the GLP-1R in canine cardiac tissue with the predicted band at ~53 kilodaltons (kDa) (**Figure 2-1 A**). Immunohistochemistry with fluorescence confocal microscopy confirmed GLP-1R expression (green) in the canine coronary vasculature and myocardium (**Figure 2-1 B and 2-1 C**). Cardiac sections were counterstained with the nuclear stain DAPI (blue) and myocardial troponin I (red) to demonstrate cellular architecture in context to localization of GLP-1R.

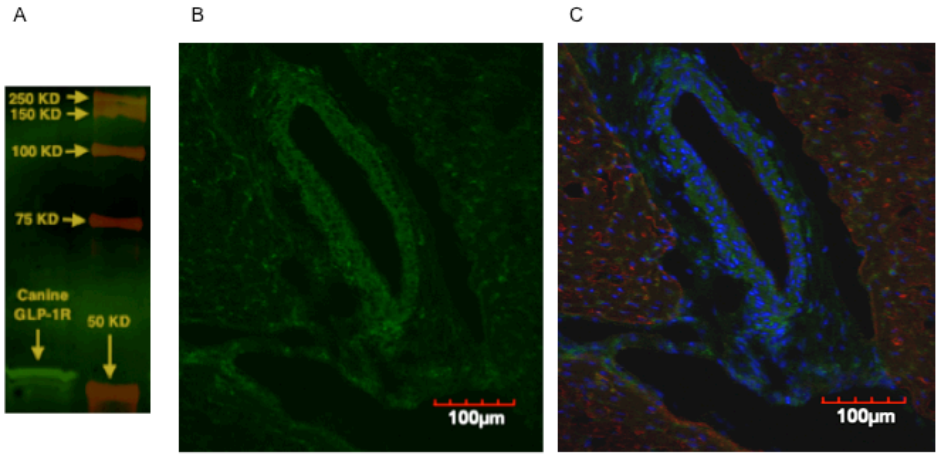

Figure 2-1 ***Cardiac and coronary expression of GLP-1R.*** High antibody selectivity for GLP-1R is demonstrated by Western Blot analysis **(A)**. Fluorescence confocal microscopy demonstrated GLP-1R expression (**green**) in both myocardium and coronary vessels **(B)**. Counter-staining of cardiac troponin I (**red**) and Nuclei (**blue**) identifies myocardial tissue and cellular architecture **(C)**.

Coronary vascular effects of GLP-1 (7-36). GLP-1 (10 pmol/L to 1 nmol/L) had no effect on isometric tension of isolated, endothelium intact and denuded

epicardial coronary rings (*n* = 5) pre-constricted with 1 μmol/L U46619 (**Figure 2-2 A**). Endothelial denudation was confirmed by a lack of relaxation to acetylcholine (10 μmol/L) and smooth muscle viability confirmed by relaxation to sodium nitroprusside (20 μmol/L). GLP-1 also had no effect on basal tension of non pre-constricted coronary arteries (data not shown). Likewise, acute intracoronary administration of GLP-1 (7-36) (10 pmol/L to 1 nmol/L) had no effect on coronary blood flow (**Figure 2-2 B**) or coronary venous PO_2 (**Figure 2-2 C**) at either CPP = 100 mmHg or 40 mmHg (*n* = 9).

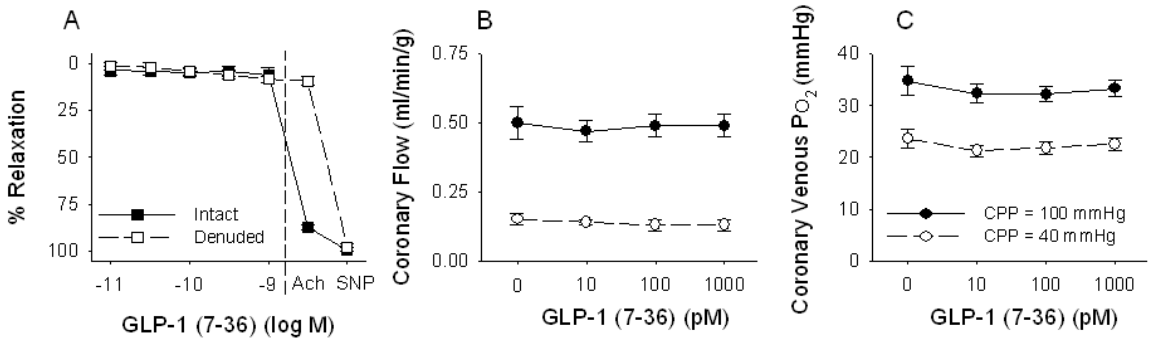

Figure 2-2 ***Direct coronary vascular effects of GLP-1 (7-36).*** **GLP-1 (7-36) had no effect on isometric tension of intact or endothelial denuded canine coronary artery rings preconstricted with U46619 (1 μM).** Denudation was confirmed by a lack of responsive to Acetylcholine (Ach; 10 μM), and viability confirmed by relaxation to sodium nitroprussside (SNP; 20 μM) **(A)**. Intracoronary infusion of GLP-1 (7-36), 10 pmol/L to 1nmol/L, had no effect on coronary blood flow **(B)** or coronary venous PO_2 **(C)** at CPP=100 mmHg or 40 mmHg.

Effects of GLP-1 (7-36) on global and regional indices of cardiac function. Hemodynamic data on the effects of GLP-1 in normal and ischemic hearts are provided in **Table 2-1**. A representative tracing of key hemodynamic data before and during GLP-1 (1 nmol/L) administration at CPP = 100 and 40 mmHg is shown in **Figure 2-3**. In these experiments we used a servo-controlled roller

pump system to maintain perfusion pressure constant at 100 mmHg and 40 mmHg. This servo-controlled system maintains pressure at these set values by alternating the frequency of a roller pump. Therefore, the phasic nature observed in coronary flow is not reflective of the cardiac cycle but due to the disruption of flow by the pump rollers (**Figure 2-33**). Acute intracoronary administration of GLP-1 dose-dependently decreased stroke volume and cardiac output at CPP = 100 mmHg. Although heart rate tended to be higher (P = 0.07), mean arterial pressure and the maximal rate of LV pressure development and relaxation (+dP/dt max and –dP/dt min) were unaffected by GLP-1. Reduction of CPP to 40 mmHg decreased stroke volume and cardiac output (P < 0.05) but did not alter blood pressure or heart rate relative to untreated control conditions at CPP = 100 mmHg. GLP-1 had no effect on blood pressure, heart rate or indices of global cardiac function at CPP = 40 mmHg. Indices of regional cardiac contractile function in the LAD perfusion territory were also unaffected by intracoronary administration of GLP-1 (7-36) at either CPP = 100 or 40 mmHg (**Figure 2-4**).

Table 2-1 *Dose-dependent effects of GLP-1 (7-36) on global indices of cardiac function.*

[GLP-1] (pmol/L)	MBP (mmHg)	HR (bpm)	+dP/dt max (mmHg/s)	-dP/dt min (mmHg/s)	CO (L/min)	SV (ml)
Coronary Perfusion Pressure = 100 mm Hg						
Baseline	80 ± 4	88 ± 6	1425 ± 216	-1102 ± 197	1.6 ± 0.2	18 ± 2
10	82 ± 3	92 ± 7	1379 ± 216	-1187 ± 194	1.5 ± 0.2	16 ± 2
100	83 ± 3	99 ± 9	1350 ± 181	-1161 ± 168	1.4 ± 0.1	14 ± 1*
1000	80 ± 4	99 ± 8	1310 ± 196	-1004 ± 146	1.3 ± 0.1*	13 ± 1*
Coronary Perfusion Pressure = 40 mm Hg						
Baseline	80 ± 2	89 ± 8	1228 ± 174	-974 ± 207	1.2 ± 0.1 †	13 ± 1 †
10	81 ± 1	93 ± 8	1182 ± 233	-981 ± 218	1.2 ± 0.1	12 ± 1
100	84 ± 2	94 ± 7	1280 ± 199	-1080 ± 226	1.2 ± 0.1	12 ± 1
1000	84 ± 1	96 ± 8	1232 ± 228	-1035 ± 207	1.1 ± 0.2	11 ± 2

Data are mean ± SE from n = 9 dogs. * $P < 0.05$ vs. baseline, same CPP. † $P < 0.05$ vs. baseline, CPP = 100 mmHg.

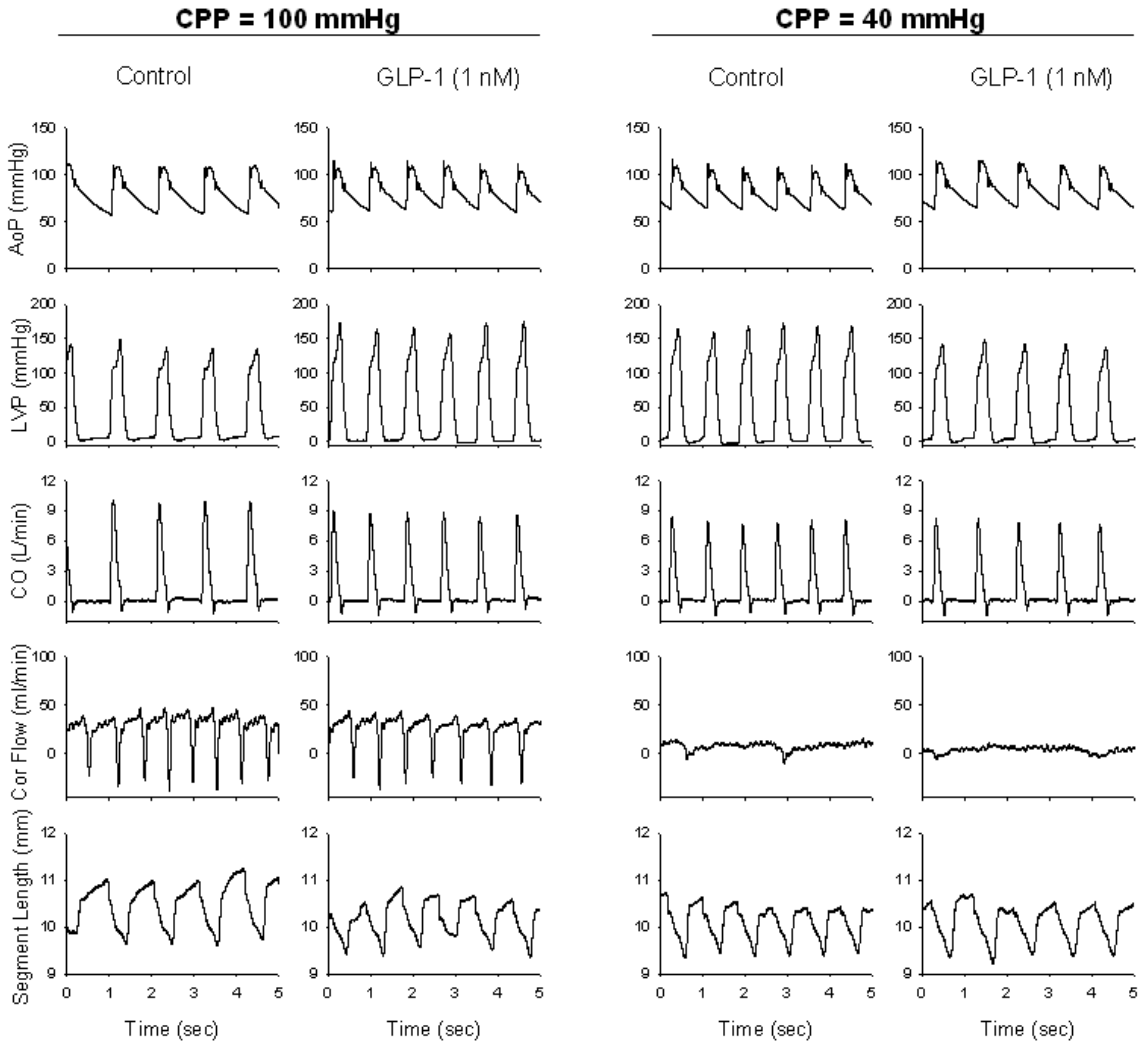

Figure 2-3 Example of original recordings of aortic pressure (AoP), left ventricular pressure (LVP), cardiac output (CO), coronary blood flow (Cor Flow), and segment length with and without intracoronary GLP-1 (7-36) (1 nmo/L) at coronary perfusion pressures (CPP) of 100 and 40 mmHg from a single canine.

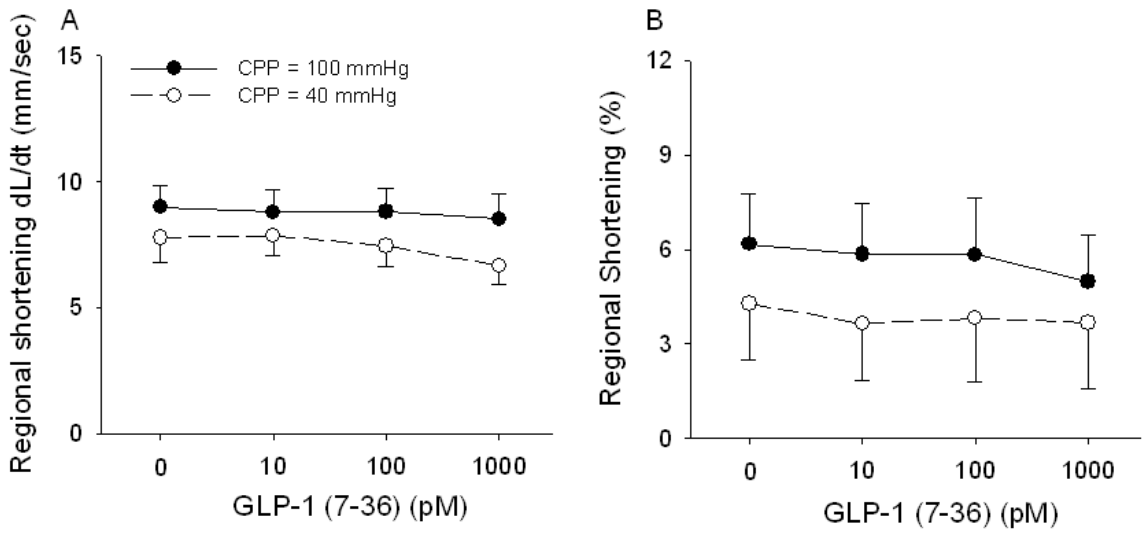

Figure 2-4 ***Direct effects of GLP-1 (7-36) on indices of regional cardiac function.*** Intracoronary infusion of GLP-1 (10 pmol/L to1 nmol/L) had no effect on the rate **(A)** or degree **(B)** of regional myocardial shortening at CPP = 100 mmHg or 40 mmHg.

Effects of GLP-1 (7-36) on myocardial substrate metabolism. At CPP = 100 mmHg, intracoronary administration of GLP-1 (7-36) (10 pmol/L to 1 nmol/L) had no effect on MVO_2, myocardial lactate uptake, glucose uptake, or glucose extraction (**Figure 2-5**). In untreated hearts, reducing CPP to 40 mmHg resulted in the expected decrease in MVO_2 (**Figure 2-5A**) and myocardial lactate uptake (**Figure 2-5B**). Administration of GLP-1 (7-36) dose-dependently augmented glucose uptake at CPP = 40 mmHg (**Figure 2-5C**), increasing consumption by ~55% from baseline (0.11 ± 0.02 μmol/min/g) to the highest 1 nmol/L dose of GLP-1 (0.17 ± 0.04 μmol/min/g). Myocardial glucose extraction was also increased ~85% at the highest dose of GLP-1 at CPP = 40 mmHg (**Figure 2-5D**). These changes in myocardial substrate metabolism induced by GLP-1 at CPP = 40 mmHg did not significantly alter MVO_2 relative to untreated baseline conditions (**Figure 2-5A**).

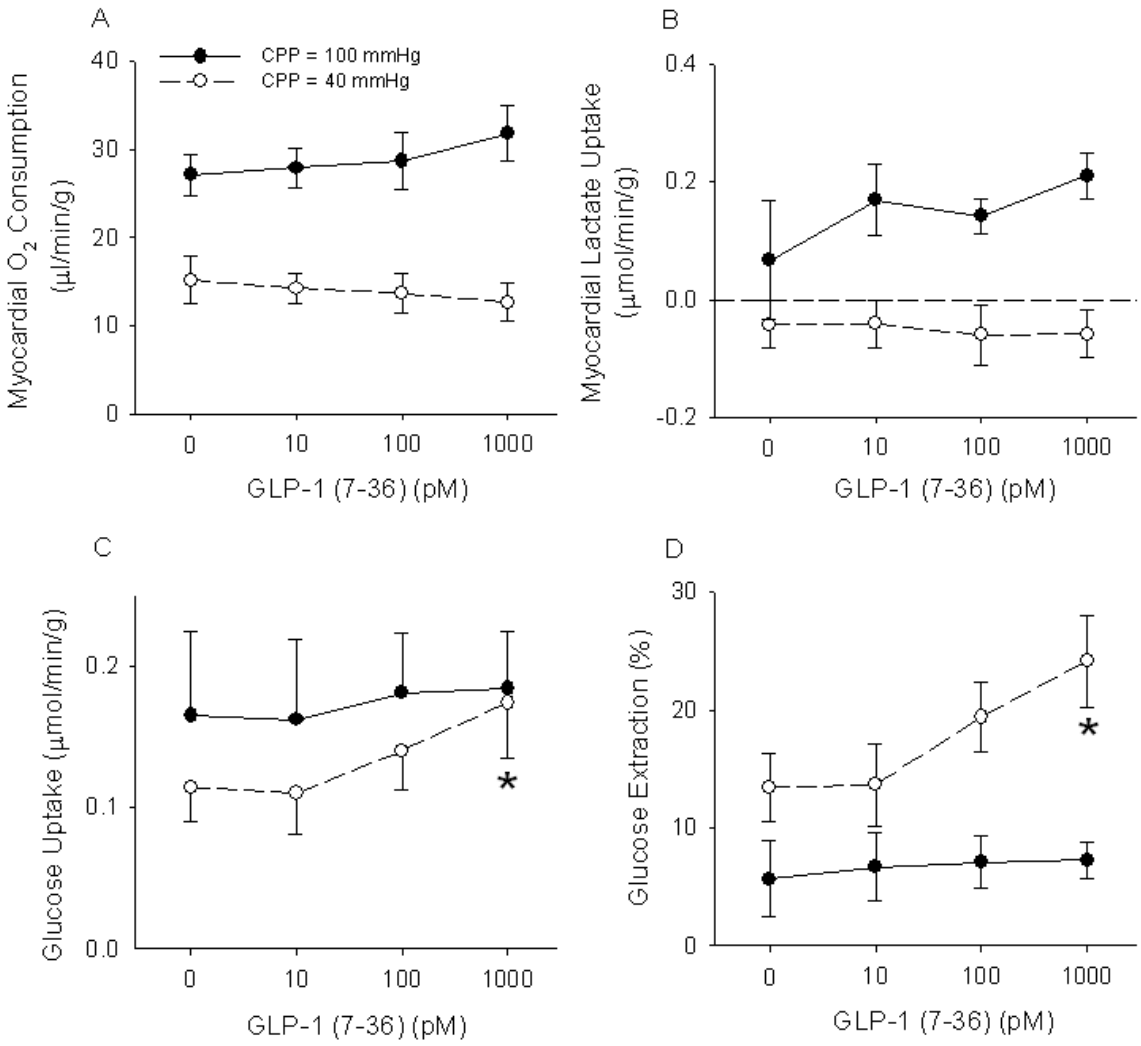

Figure 2-5 ***Direct dose-dependent effects of GLP-1 (7-36) on myocardial metabolism*****.** GLP-1 did not effect myocardial oxygen consumption **(A)**, or lactate uptake **(B)** at CPP=100 mmHg or 40 mmHg. GLP-1 (7-36) dose dependently increased myocardial glucose uptake **(C)** and extraction **(D)** at CPP=40 mmHg, but had no effect at CPP=100 mmHg. * $P < 0.05$ vs. baseline at the same CPP.

Discussion

The purpose of this investigation was to evaluate the direct coronary and cardiac effects of GLP-1 (7-36) administration *in-vivo*. We tested the hypothesis that acute, intracoronary administration of GLP-1 (7-36) augments coronary blood flow, myocardial glucose metabolism and cardiac contractile function in

normal and/or ischemic hearts in a dose-dependent manner. The rationale was based on earlier studies indicating that systemic infusions of GLP-1 result in increased LV contractile function and myocardial glucose metabolism at baseline (93), and in conditions of cardiac stress (i.e. coronary artery occlusion (88, 90), pacing induced heart failure (94, 110). In addition, GLP-1 has also been shown to induce endothelium-dependent vasodilation of isolated systemic arteries (131), and to increase coronary blood flow *in-vitro* (91, 93), and *in-vivo* (89). However, whether these effects of GLP-1 are mediated by direct actions on the heart and coronary circulation independent of changes in MVO_2, and/or by other indirect peripheral/central effects has not been established. Novel findings of the current study were that acute, intracoronary administration of GLP-1 (7-36): 1) did not directly affect coronary blood flow *in-vivo* or vascular tone *in-vitro*; 2) modestly reduced stroke volume and cardiac output at CPP = 100 mmHg without affecting arterial pressure; 3) did not influence MVO_2, global or regional contractile function, or cardiac lactate uptake at CPP = 100 or 40 mmHg; 4) significantly increased myocardial glucose uptake and extraction, but only at CPP = 40 mmHg. We also confirmed GLP-1R expression in the coronary vasculature and myocardium of canines. Taken together, these findings indicate that acute (~5 to 10 min), intracoronary administration of GLP-1 (7-36) preferentially augments glucose metabolism in ischemic myocardium, independent of effects on cardiac contractile function or coronary blood flow.

Coronary and cardiac expression of GLP-1R. Data from the present investigation confirm expression of GLP-1R in both the coronary vasculature and

myocardium. While previous studies have demonstrated expression of GLP-1R in canine myocardium (94), mouse heart and coronary vasculature (93), and isolated human coronary artery endothelial cells (143), we are the first group to demonstrate relative GLP-1R expression in canine coronary vasculature and myocardium. We observed GLP-1R expression in all layers of coronary arterial wall, including the endothelium and adventitia. Our results indicate GLP-1R has more uniform and ubiquitous expression in canine myocardium and coronary circulation (**Figure 2-1B**) than was previously shown in mice. Importantly, selective binding of the GLP-1R antibody used in this investigation at the specified protein band (53 kDa) was confirmed using Western Blot (**Figure 2-1A**).

Coronary vascular effects of GLP-1 (7-36). Our findings demonstrate that although GLP-1R is expressed in the coronary vasculature (**Figure 2-1B**), acute administration of GLP-1 does not directly modulate coronary vascular tone *in-vitro* (**Figure 2-2A**) or *in-vivo* (**Figure 2-2B**). These data are in contrast with earlier studies which documented that GLP-1 induces endothelium-dependent vasodilation in isolated arteries, increases coronary blood flow in isolated hearts under normal conditions and during reperfusion, and following pacing induced cardiomyopathy in canines. However, such previous studies were conducted in aorta (130, 132), femoral (129), and pulmonary vessels (131), isolated rodent hearts (91, 93), and with systemic administration of recombinant GLP-1 (7-36) in canines. It is also important to recognize that the increased coronary blood flow previously documented *in-vivo* in canines was accompanied by increases in

cardiac contractile function and MVO_2 (89), the primary determinants of coronary flow (38). Thus, the effects of GLP-1 on coronary blood flow in those experimental conditions could have been mediated by direct effects on the vasculature and/or by indirect effects via metabolic vasodilation secondary to increased MVO_2. The reasons for the discrepant findings regarding the vascular effects of GLP-1 are unclear but are likely related to differences in specific vessels studied, species investigated and/or dose and route of GLP-1 administration. Regardless, our findings provide strong evidence that acute administration of physiologic/pharmacologic concentrations of GLP-1 directly in to the coronary circulation does not influence coronary vasomotor tone.

Cardiometabolic effects of GLP-1 (7-36). Earlier studies indicate that GLP-1 augments myocardial glucose metabolism under normal and post-ischemic conditions in isolated hearts (91, 93), and following cardiac ischemia and/or dilated cardiomyopathy *in-vivo* (89, 94, 110). However, we found that intracoronary administration of GLP-1 (7-36) preferentially and dose-dependently augments myocardial glucose metabolism during ischemia (CPP = 40 mmHg), but not during normal perfusion (CPP = 100 mmHg) (**Figure 2-5**). This finding is consistent with previous data indicating that GLP-1 augments myocardial glucose uptake under conditions of cardiac stress (e.g. ischemia/reperfusion injury, dilated cardiomyopathy) (89, 94, 110). Data from the current investigation extend this finding and demonstrate that acutely increasing coronary plasma GLP-1 concentration to ~1 nmol/L increases glucose uptake of ischemic myocardium by ~55%. Importantly, this effect was concentration dependent and differed from

other studies, which found that direct coronary administration of GLP-1 increases glucose uptake under normal conditions in isolated hearts (91, 93). Our results indicate that longer, systemic administration of GLP-1 is not mandatory for the insulinomimetic effect to be manifest and argue against peripheral/central actions of GLP-1 driving alterations in substrate metabolism. Whether the effect of GLP-1 (7-36) on ischemic myocardial glucose metabolism is dependent on GLP-1R activation and/or actions of the (9-36) degradation product merits further investigation.

Effects of GLP-1 (7-36) on regional and global indices of cardiac function. Several previous investigations have reported that GLP-1 increases indices of LV function (i.e. developed pressure, wall thickening, dP/dt and ejection fraction) during reperfusion, heart failure and dilated cardiomyopathy (88-90, 93, 94, 110). However, we observed no direct effect of acute intracoronary GLP-1 (7-36) infusion on indices of regional (**Figure 2-4**) or global cardiac contractile function during ischemia (**Table 2**). This finding is in contrast to our hypothesis that increases in glucose metabolism would improve contractile function in ischemic myocardium, as glucose has a high P/O ratio (ATP produced per oxygen consumed) and earlier data support that increased myocardial glucose metabolism improves cardiac efficiency during ischemia (52, 53, 112, 118-120). In particular, previous studies from our group documented that insulin-mediated increases in myocardial glucose metabolism significantly improve regional cardiac function in ischemic canine hearts (CPP = 40 mmHg) (52, 57). However, the increase in myocardial glucose uptake induced by insulin in these studies

(~0.50 μmol/min/g) was substantially higher than that reported in the present study (~0.20 μmol/min/g).

Interestingly, we did observe a moderate dose-dependent decrease in stroke volume and cardiac output at normal perfusion pressure (CPP = 100 mmHg), without corresponding changes in dP/dt or aortic pressure (**Table 2**). This reduction in cardiac output was associated with an ~10 beats/min increase in heart rate. Although there were no changes in dP/dt in our experiments, the reductions in stroke volume and cardiac output suggest GLP-1 reduced contractile performance. This observation is supported by a previous study in which recombinant GLP-1 (7-36) decreased LV developed pressure and dP/dt when infused into the coronary circulation of isolated rat hearts (91). Taken together, these findings indicate that direct (intracoronary) cardiac effects of GLP-1 differ from indirect (peripheral/central) effects.

Limitations of the study. It is possible that that the assessment of regional myocardial substrate uptake and contractile function can be influenced by contamination of interventricular venous drainage by blood from non-LAD perfused myocardium and/or by collateral flow to the hypoperfused LAD perfusion territory. Importantly, an earlier study by Vinten-Johansen et al. conducted using systemically administered radioactively-labeled red blood cells demonstrated that blood taken from the anterior interventricular vein is almost exclusively from LAD perfused myocardium, even at CPP = 40 mmHg (144). Our laboratory also previously demonstrated that collateral flow to the LAD at a CPP of 40 mmHg is negligible (~0.01 ± 0.01 ml/min/g) (57). This finding is supported

by marked reduction of cardiac performance observed when CPP was lowered to 40 mmHg (**Table 2-1**). Thus, our measurements of myocardial substrate metabolism computed from arteriovenous differences and LAD flow were likely not significantly affected by collateral flow or venous contamination. Further, any contamination of venous drainage would have resulted in dilution of the measures, and actually served to diminish our overall treatment effect on cardiac metabolism. Therefore, if such effects were present, the ~55% increase in glucose uptake would be an underestimate of the effect of GLP-1 in ischemic myocardium (**Fig 2-5C**). While our studies examined the acute effects of intracoronary GLP-1 (7-36) on myocardial glucose, lactate and oxygen uptake, additional studies to also examine the effects of GLP-1 on the fates of other substrates such as fatty acids, triglycerides and myocardial glycogen stores are needed.

GLP-1 infusions in this investigation were conducted after hemodynamic variables had stabilized at CPPs of 100 and 40 mmHg. Although it is possible that hemodynamic status could have changed over time, the relatively constant measures of coronary blood flow, cardiac function and MVO_2 at each of these pressures argues for stability of the experimental preparation over time.

Our observed regional segment shortening data were lower than what is typically reported in the literature (141, 145). These low values are likely related to differences in depth of crystal placement and/or local cardiac fiber orientation at the depth in which the crystals were placed. These aspects of crystal implantation environment are not controlled under the present experimental

protocol. It is also possible that the low observed values for regional segment shortening are due to myocardial stunning subsequent to cannulation of the LAD. However, we carefully monitor all hemodynamic variables before and following cannulation to ensure that these parameters return to baseline values prior to beginning the experimental protocol. These variables typically return to normal within ~2 min of reperfusion and we allowed at least a 10 minute recovery period to ensure adequate recovery time. Regardless of the signal magnitude, we did observe expected reductions in regional function during ischemia, and importantly GLP-1 did not affect segment shortening at normal coronary perfusion pressure (CPP = 100 mmHg) or during ischemia (CPP = 40 mmHg).

Conclusion

Data from this investigation indicate that acute, intracoronary infusion of GLP-1 (7-36) preferentially augments glucose metabolism in ischemic myocardium, independent of effects on coronary blood flow. These findings demonstrate that the salutary effects of GLP-1 on myocardial glucose metabolism occur dose-dependently within minutes of intracoronary administration during ischemia. Thus acute cardiac administration of GLP-1 may be useful in modulating cardiac metabolism acutely in a setting where ischemia is anticipated, such as elective cardiac surgery. Our data also indicate that although GLP-1R is expressed in myocardium and coronary circulation, GLP-1 (7-36) does not affect coronary vasomotor tone or coronary flow when administered directly into canine coronary vasculature over an ~20 min time period. Further investigations to uncover direct and systemic mechanisms by which GLP-1

based treatments affect cardiac function and metabolism will be useful for determining the best strategies to optimize therapeutic utilization of these compounds.

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Chapter 3

Impaired cardiometabolic responses to glucagon-like peptide 1 in Metabolic Syndrome and Type 2 Diabetes Mellitus

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Abstract

This study examined the hemodynamic and cardiometabolic effects of intravenous glucagon-like peptide 1 (7-36) (GLP-1; 1.5 pmol/kg/min) in lean and metabolic syndrome (MetS) swine, and in lean and MetS/Type 2 Diabetic (T2D) humans. Under fasting conditions, GLP-1 did not affect blood pressure, heart rate, or coronary blood flow in humans or swine. In lean humans, GLP-1 increased MGU over 4-fold (P = 0.03) and increased MVO_2 by ~20% (P = 0.03). However, these effects were absent in obese humans with Type 2 diabetes mellitus. In lean exercising swine, GLP-1 augmented the myocardial glucose uptake (MGU) response to increasing MVO_2 (P = 0.01). These effects of GLP-1 were absent in MetS swine. Western blotting and immunohistochemistry demonstrated no differences in GLP-1 receptor content between lean and MetS swine in coronary (P = 0.95) or cardiac (P = 0.46) tissue. In myocardial tissue slices exposed *ex-vivo*, GLP-1 (1 to 5 nmol/L) dose dependently increased p38-MAPK activity in lean (P =0.04) but this effect was absent in MetS swine. GLP-1 did not affect myocardial PKA activity in either group. These data indicate that cardiometabolic responses to GLP-1 (7-36) are impaired in the setting of MetS and T2DM via diminished activation of p38-MAPK, without alterations in GLP-1 receptor expression or PKA-dependent signaling.

Introduction

The increasing prevalence of Type 2 Diabetes Mellitus (T2DM) is a major health concern in the United States and globally (2, 9). Once considered largely endemic to Western societies, T2DM has become an epidemic for many regional

and cultural sectors worldwide (1). Other major health concerns such as obesity, hypertension, and dyslipidemia are often associated with hyperglycemia or overt T2DM (5, 146). Clusters of these risk factors are collectively described as the metabolic syndrome (MetS) (7, 147, 148). Patients with MetS and/or T2DM carry a significantly increased burden of cardiovascular disease (3, 10, 149). Approaches to restore the balance between myocardial oxygen delivery and metabolism during ischemia, i.e. improving myocardial oxygen supply and promoting more efficient cardiac substrate utilization, are currently being investigated as a means to improve the treatment and management of obesity-related cardiovascular disease (14, 20, 26, 53, 150-152).

Advancements in the treatment of T2DM include drug therapies designed to augment actions of the incretin system, in particular the actions of glucagon-like peptide 1 (GLP-1) (153-174). GLP-1 is released from L-cells of the small intestine in response to feeding and is an agonist of the G protein-coupled receptor (GLP-1R) (134, 175). In pancreatic beta cells, this ligand/receptor interaction causes glucose-dependent increases in cyclic adenine monophosphase (cAMP) and phosphokinase A (PKA) activity, leading to increased glucose-stimulated insulin secretion (58, 59). GLP-1 based therapies are currently prescribed for systemic glucose management, and have recently come under investigation for potential cardioprotective actions (176-179). Several studies have revealed that intravenous administration of GLP-1 significantly reduces myocardial infarct size following ischemia/reperfusion injury and improves cardiac contractile function in the settings of coronary artery disease,

hear failure, and following myocardial ischemia (87-90, 94, 98, 110, 180, 181). While the mechanisms responsible for these effects of GLP-1 are not yet fully understood, increases in myocardial glucose uptake and coronary blood flow via a p38 mitogen-activated protein kinase (MAPK) and/or PKA dependent signaling pathway have been implicated (91, 93, 94, 101, 182). Although recent evidence suggests that the insulinotropic effects of GLP-1 are attenuated in the setting of MetS and T2DM (95, 183), data on the cardiovascular effects of GLP-1 in MetS or T2DM are critically lacking.

The goal of the present investigation was to examine whether MetS or T2DM influence the cardiovascular effects of GLP-1. To accomplish this goal, we performed studies in lean vs. MetS/T2DM humans and in lean vs. obese/MetS Ossabaw swine to examine the hypothesis that the myocardial actions (substrate metabolism and perfusion) of GLP-1 are impaired in the settings of MetS/T2DM. We further evaluated whether differences arose due to alterations in GLP-1 receptor expression and/or downstream signaling through cAMP/PKA or p38-MAPK pathways. Data from these studies provide the first evidence of defects in the functional metabolic effects of GLP-1 *in-vivo* and *in-vitro* in obesity/MetS and T2DM.

Methods

The human studies and protocols performed were approved by the Indiana University Institutional Review Board and conducted in accordance with the Declaration of Helsinki. Human subjects provided written informed consent for these investigations. The procedures and protocols performed in swine 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.

Human subjects inclusion criteria. All human subjects were between the ages of 18 and 50 years. Lean subjects were defined as having a BMI < 25 kg/m^2, in good general health, and taking no regular medications. Diabetic subjects were selected on the following basis: obese (BMI > 30 kg/m^2 but < 40 kg/m^2), HbA1c 7.0 to 10.0%, and treated with diet and exercise plus oral agents or injected insulin.

Human subjects exclusion criteria. Human subjects were excluded based on the following criteria: chronic illness (other than T2DM) or infection, known coronary artery disease or abnormal ECG on screening evaluation, blood pressure > 160/100 mmHg on two occasions during screening evaluations, total cholesterol > 240 mg/dL, treatment with GLP-1 agonist or DPP4 inhibitor within 6 months, known intolerance to injected GLP-1 agonist, treatment with PPAR-γ agonist within 6 months, recognized microvascular complications (retinopathy, nephropathy, neuropathy), unwillingness or inability to use injected insulin, inability to remain supine and comfortable for the duration of the PET protocol, known or planned radiation exposure that would result in greater than 500 mrem total exposure in a contiguous 12 month period, and/or current pregnancy.

Preparation of human subjects with T2DM. All human subjects with T2DM were placed on a uniform medication regimen for glucose management consisting of only glargine (Sanofi Aventis, Bridgewater, NJ, USA) and lispro (Eli Lilly and Company, Indianapolis, IN, USA) insulins two weeks prior to the

scheduled PET study. The night prior to PET studies blood samples were collected (minimum of 4 hours from the most recent meal) and hemodynamic parameters were measured, then subjects used their current meal bolus and nighttime basal insulin dosing to provide basal control of fasting sugar until completion of the PET protocol the following morning. In one subject, an overnight infusion of regular insulin (Eli Lilly and Company, Indianapolis, IN, USA) was used to achieve adequate fasting glucose overnight; this was discontinued for the subsequent PET scanning procedure, using only insulin glargine for ongoing glucose control.

Human subjects study protocol. All studies were conducted with subjects in the fasting resting state. Subjects were admitted to the Clinical Research Center for a standardized meal the evening prior to the planned study. Two intravenous access lines were placed: A 20G X 3cm Teflon coated catheter in the antecubital vein of one arm for initial blood sampling, as well as GLP-1 and PET tracer infusions, and the following morning a second 20G X 3cm Teflon coated catheter was placed in the contralateral arm for blood sampling. Prior to an overnight infusion of normal saline or GLP-1 (1.5 pmol/kg/min, iv, Bachem Inc., Torrance, CA, USA), subjects had venous blood samples collected for measurement of relevant metabolites (glucose, insulin, free fatty acids, GLP-1 (7-36), and total GLP-1). Non-invasive measurements of hemodynamics were taken at the same time point using surface electrodes placed on the thorax and neck (impedence cardiography, CardioDynamics, San Diego, CA, USA), and an automated blood pressure cuff. Positron Emission Tomography (PET)

measurements began in the morning following an overnight (10 hrs) test infusion, and lasted ~3 hours. These measurements include cardiac perfusion and oxidative metabolism (^{11}C-acetate) and glucose uptake (^{18}F-deoxyglucose) under resting fasting conditions. Following PET imaging, end-of-protocol blood samples and hemodynamic measures were taken (~13 hrs total infusion time), and test infusions was terminated.

PET modeling and analysis. Myocardial glucose uptake was calculated by analyzing ^{18}F-deoxyglucose kinetics using the standard three-compartment model. A two-compartment model was applied to metabolite-corrected ^{11}C-acetate data to derive rates of cardiac perfusion (early influx kinetics) and myocardial oxygen consumption (MVO_2) (late disappearance kinetics)(184).

Ossbaw swine model of MetS. Lean control swine were fed ~2200 kcal/day of standard chow (5L80, Purina Test Diet, Richmond, IN, USA) containing 18% kcal from protein, 71% kcal from complex carbohydrates, and 11% kcal from fat. MetS swine were fed an excess ~8000 kcal/day high fat/fructose, atherogenic diet containing 16% kcal from protein, 41% kcal from complex carbohydrates, 19% kcal from fructose, and 43% kcal from fat (mixture of lard, hydrogenated soybean oil, and hydrogenated coconut oil), and supplemented with 2.0% cholesterol and 0.7% sodium cholate by weight (KT324, Purina Test Diet, Richmond, IN, USA). Both lean and MetS castrated male swine were fed their respective diets for ~16 weeks prior to surgical instrumentation.

Swine surgical instrumentation. Ossabaw swine were fasted overnight prior to surgical procedures. Sedation was initiated with a combination of telazol (5 mg/kg sc) and xylazine (2.2 mg/kg sc). Following endotracheal intubation, a surgical plane of anesthesia was maintained by mechanical ventilation with 1 to 3% isoflurane supplemented with oxygen. Using sterile techniques, a left lateral thoracotomy was performed in the 5th intercostal space, and a catheter (17-Ga pressure-monitoring catheter, Edwards LifeSciences, Irvine, CA, USA) was implanted in the descending thoracic aorta to measure aortic blood pressure and heart rate, and obtain arterial blood samples. A second catheter was placed in the anterior interventricular vein for coronary venous blood sampling from the LAD perfusion territory and iv drug administration. The proximal left anterior descending (LAD) coronary artery was secured to a Transonics perivascular flow transducer (Transonic Systems, Inc., Ithaca, NY, USA) for coronary blood flow measurements. Catheters and the flow transducer wires were tunneled subcutaneously and exteriorized between the scapulae. The chest was closed in layers, and the pneumothorax evacuated with a chest tube using manual suction. Antibiotics (exceed, 5 mg/kg, im), anti-inflammatories (rimadyl, 4 mg/kg, im), and analgesics (buprenorphine, 0.015 mg/kg, im) were administered to prevent infection and manage post-operative pain. The externalized catheters/wire were protected with Vetrap (Animal Care Products, St. Paul, MN, USA) and custom made jackets. An elastomeric balloon pump with flow control tubing (MILA International, Erlanger, KY, USA) was connected to the coronary venous catheter for continuous infusion of heparinized saline (2.5 U/ml at 0.5 ml/hr), and for GLP-

1 (7-36) administration. The aortic catheter was cleared, flushed and filled with heparinized saline (5 U/ml) daily.

Swine experimental protocol and blood sampling. Following recovery from surgery, experiments were conducted in lean and MetS Ossabaw swine at rest and during graded treadmill exercise, before and during the administration of GLP-1 (7-36) (1.5 pmol/kg/min, iv, started 2 hrs. prior to exercise protocol). Importantly, each animal served as its own control. Aortic blood pressure, heart rate and LAD coronary blood flow were continuously recorded at rest and during two levels of treadmill exercise (~2 mph and ~5 mph). Each exercise period lasted ~2 min, and the swine were rested between bouts until hemodynamic parameters returned to baseline values. Baseline blood samples were collected before and after the 2 hrs continuous infusion of GLP-1 (7-36) for phenotypic profiling and measurement of plasma GLP-1. Arterial and anterior interventricular coronary venous samples were also simultaneously collected at rest and during exercise in heparinized syringes and immediately sealed and placed on ice. These blood samples were taken when hemodynamic variables had stabilized at each level and analyzed in duplicate for hematocrit, PCO_2, PO_2, oxygen content, lactate, pH and glucose with a GEM Premier 3000 blood gas analyzer and 682 Co-oximeter system (Instrumentations Laboratories, Bedford, MA, USA). Myocardial oxygen consumption (MVO_2, μmol O_2/min/g) was calculated by multiplying coronary blood flow by the arterial coronary venous difference in oxygen content.

Western blot analysis. Following excision of the hearts, the coronary circulation was perfused with physiologic salt solution to removed blood and blood proteins, then crude transmural LV samples and epicardial coronary vessels were quickly isolated, frozen in liquid N_2 and stored at -80˚C. These samples were later homogenized in lysis buffer, and protein was quantitated using a DC protein assay. 25 µg of protein was loaded onto 7% acrylamide gels, electrically separated and then transferred overnight onto nitrocellulose membranes. Membranes were blocked for one hour prior to an overnight incubation in primary antibody at 4˚C. Membranes were exposed to secondary antibodies for 1 hr at ambient temperature. Immunoreactivity and quantitation was determined with a Li-Cor Odyssey system (Li-Cor Biosciences, Lincoln, NE, USA). Intensities were normalized to that of α-actin (691002, MP Biomedicals, Solon, OH, USA), and band size was determined using a standard protein ladder (161-0375, BIO Rad, Hercules, CA, USA). Total p38-MAPK was determined with a p38α-MAPK antibody (9218, Cell Signaling Technologies, Boston, MA, USA), and GLP-1 receptor content determined using a polyclonoal antibody (ab39072, abcam, Cambridge, MA, USA).

Immunohistochemistry and confocal microscopy. Fresh LV cardiac samples were snap frozen in liquid N_2 and stored at -80˚C. The frozen samples were embedded in Tissue-Tek® OCT™ (Sakura Finetek USA Inc, Torrance, CA, USA) and sliced into 10 um sections. Slices were incubated in mouse monoclonal antibodies against cardiac troponin I (ab10231; 1:1,000), 4',6-diamidino-2-phynlindole (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 (Olympus America Inc., Center Valley, PA, USA).

Enzyme activity assays. Fresh transmural LV tissue slices (~200 μm) from lean and MetS Ossabaw swine hearts were placed into preheated (37˚C) tissue baths of physiological salt solution and oxygenatated with 95% O_2 and 5% CO_2. The baths were then either dosed with GLP-1 (7-36) to bring the concentration to 1 nmol/L or 5 nmol/L, or not dosed for a sham control. The tissue slices were incubated in these conditions for 1 hr. at which time they were quickly rinsed and snap frozen in liquid N_2.

The p38-MAP Kinase enzyme activity was determined using the protocol provided with the nonradioactive p38-MAP Kinase Assay Kit (9820, Cell Signaling Technologies). Briefly, frozen tissue sample were homogenized in lysis buffer, protein content quantitated then adjusted with the addition of lysis buffer to equalize protein concentrations (0.875 μg/ul). 20 μl of immobilized Phospho-p38-MAPK (Thr180/Tyr182) primary antibody bead slurry was added to 200 μl protein extracts and incubated overnight at 4˚C. This mixture was then centrifuged to isolate the bead pellet and washed with lysis buffer and kinase buffer, two times each. The pellet was then suspended in 50 μl of kinase buffer supplemented with 200 μmol/L ATP and 1 μL ATF-2 fusion protein (kinase substrate), and incubated in a 30˚C water bath for 30 min. This reaction was

terminated with 3X SDS running buffer, heated at 95˚C for 5 min. and then 30 µl loaded onto 10% acrylamide gels. A biotenylated protein marker (7727, Cell Signaling Technology) was used to estimate molecular weights. After transferring to a nitrocellulose membrane, the membranes were treated with blocking buffer for 1 hr then incubated in Phospho-ATF-2 (Thr 71) primary antibody (1:1,000) at 4˚C overnight. The membranes were incubated in a secondary antibodies conjugated with HRP at ambient temperature for 1 hr, incubated in HRP substrate for 3 minutes, and imaged to determine immunoreactivity. Signal quantitation was conducted using image J, and normalized to sham controls.

PKA enzyme activity assays were conducted in accordance with the nonradioactive MESACUP® Protein Kinase Assay Kit (5230, MBL international, Woburn, MA). Breifly, 12 µl of protein samples (0.875 µg/µl) was added to 108 µl of a reaction mixture with and without supplemental cAMP (20 µmol/L) to determine maximal and basal activity respectively. These mixtures were incubated in PS-peptide coated wells in a water bath at 25˚C for 10 minutes. The reaction was terminated with a stop solution and the wells washed several times. 100 µl of biotinylated antibody was then added to each well and incubated at 25˚C for 1 hr The wells were again washed several times then treated with 100 µl of POD-conjugated streptavidin at 25˚C for 1 hr. After a final wash, 100 µl of colorimetric substrate was added for 5 minutes, and then terminated with 100 µl of stop solution. Optical density was measured at 492 nm, and values were normalized to sham controls with and without cAMP added to the reaction mixtures.

Statistical analysis. Data are presented as mean ± SE. For all statistical comparisons, $P \leq 0.05$ was considered statistically significant. Human studies data were evaluated using a one-way Analysis of Variance (ANOVA) comparing 3 groups (lean saline, lean GLP-1 and T2DM GLP-1). To compare lean to MetS swine, or treated to untreated swine, under the same experimental conditions one-way ANOVA was used. To evaluate the effects of MetS and/or GLP-1 on hemodynamic responses to exercise, a three-way ANOVA was used (Factor A: drug treatment; Factor B: exercise level; Factor C: diet). For *in-vitro* studies, t-tests were conducted to determine the effect of GLP-1 within lean and MetS groups, and one-way ANOVA was used to compare lean to MetS groups under the same experimental condition. When significance was found with 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 response variables (myocardial glucose uptake) plotted vs. MVO_2.

RESULTS

Phenotypic and hemodynamic data in resting human subjects with and without intravenous GLP-1. Baseline fasting phenotypic data in lean and MetS/T2DM human subjects differed as expected: Body Mass Index (BMI), plasma insulin and glucose were significantly elevated in the MetS/T2DM group (**Table 3-1A**). The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was also significantly elevated in MetS/T2DM under baseline fasting conditions; however, non-esterified fatty acids were not significantly different between groups. GLP-1 (7-36) infusion (1.5 pmol/kg/min iv for ~13 hrs) increased

plasma GLP-1 (7-36) concentrations equally in lean and MetS/T2DM human subjects by ~15 fold. Total plasma GLP-1 concentration at baseline was elevated in MetS/T2DM, but was equal to that of lean controls following the ~13 hrs of GLP-1 (7-36) infusion. Administration of GLP-1 plus basal insulin overnight significantly lowered plasma glucose in subjects with MetS/T2DM; there was no effect of GLP-1 on plasma glucose in lean subjects. Plasma glucose was not different between lean and MetS/T2DM groups following GLP-1 administration. Baseline resting systolic blood pressure, mean aortic pressure, and heart rate were significantly elevated in MetS/T2DM subjects compared to lean subjects, while baseline stroke volume, cardiac output, and diastolic blood pressure were not different (**Table 3-1B**). GLP-1 administration did not affect any hemodynamic parameter measured in either lean or MetS/T2DM human subjects.

Table 3-1 A *Phenotypic characteristics of lean and T2DM human subjects before and after GLP-1 (7-36) administration (1.5 pmol/kg/min, iv, 13 hrs).*

	Lean	Lean + GLP-1	T2DM	T2DM + GLP-1
BMI (kg/m^2)	24.6 ± 3.5		36.8 ± 2.8*	
Insulin (μU/ml)	13 ± 2	11 ± 2	28 ± 6*	19 ± 4
Glucose (mg/dl)	96 ± 5	86 ± 2	121 ± 6*	91 ± 3†
HOMA-IR	3.2 ± 0.6	2.5 ± 0.5	8.8 ± 2.1*	4.2 ± 0.7
NEFA (mmol/L)	0.15 ± 0.07	0.21± 0.04	0.20 ± 0.05	0.27 ± 0.07
Total GLP-1 (pmol/L)	60 ± 9	349 ± 47†	102 ± 18*	307 ± 33†
GLP-1 (7-36) (pmol/L)	6 ± 2	85 ± 10†	5 ± 1	81 ± 11†

Values are mean ± SE for lean (n = 10) and T2DM (n = 6) human subjects. NEFA (non-esterified fatty acids). * $P \leq 0.05$ vs lean same condition, † $P \leq 0.05$ vs baseline same

Table 3-1B *Hemodynamic characteristics of lean and T2DM human subjects before and after GLP-1 (7-36) administration (1.5 pmol/kg/min, iv, 13 hrs).*

	Lean	Lean + GLP-1	T2DM	T2DM + GLP-1
Systolic Blood Pressure (mmHg)	113 ± 4	113 ± 4	134 ± 6*	141 ± 10*
Diastolic Blood Pressure (mmHg)	66 ± 5	69 ± 3	76 ± 4	82 ± 5*
Mean Aortic Pressure (mmHg)	82 ± 5	84 ± 3	96 ± 4*	104 ± 7*
Heart Rate (beats/min)	66 ± 3	65 ± 2	77 ± 3*	72 ± 3*
Cardiac Output (L/min)	6.1 ± 0.4	5.4 ± 0.3	6.5 ± 1.1	6.0 ± 0.6
Stroke Volume (ml/beat)	90 ± 7	85 ± 6	84 ± 12	84 ± 8

Values are mean ± SE for lean (n = 10) and T2DM (n = 6) human subjects.
* $P \leq 0.05$ vs lean same condition.

Effects of GLP-1 (7-36) on myocardial blood flow, glucose uptake and total oxidative metabolism in lean and MetS/T2DM human subjects. Using dynamic positron emission tomography, we determined that iv infusion of GLP-1 (7-36) increased myocardial glucose uptake over 4-fold in lean subjects. However, myocardial glucose uptake in MetS/T2DM subjects treated with GLP-1 was markedly lower than GLP-1 stimulated uptake in lean subjects and no different from lean saline treated controls (**Figures 3-1A and 3-1B**). Administration of GLP-1 did not affect basal coronary blood flow in either group (**Figure 3-1D**), but modestly increased basal MVO_2 in lean subjects (**Figure 3-1C**). Importantly, these impaired responses to GLP-1 in MetS/T2D occurred despite similar plasma concentrations of GLP-1 (total and 7-36), glucose, and non-esterified fatty acids, and despite higher circulating insulin concentrations in DM subjects (**Table 3-1A**).

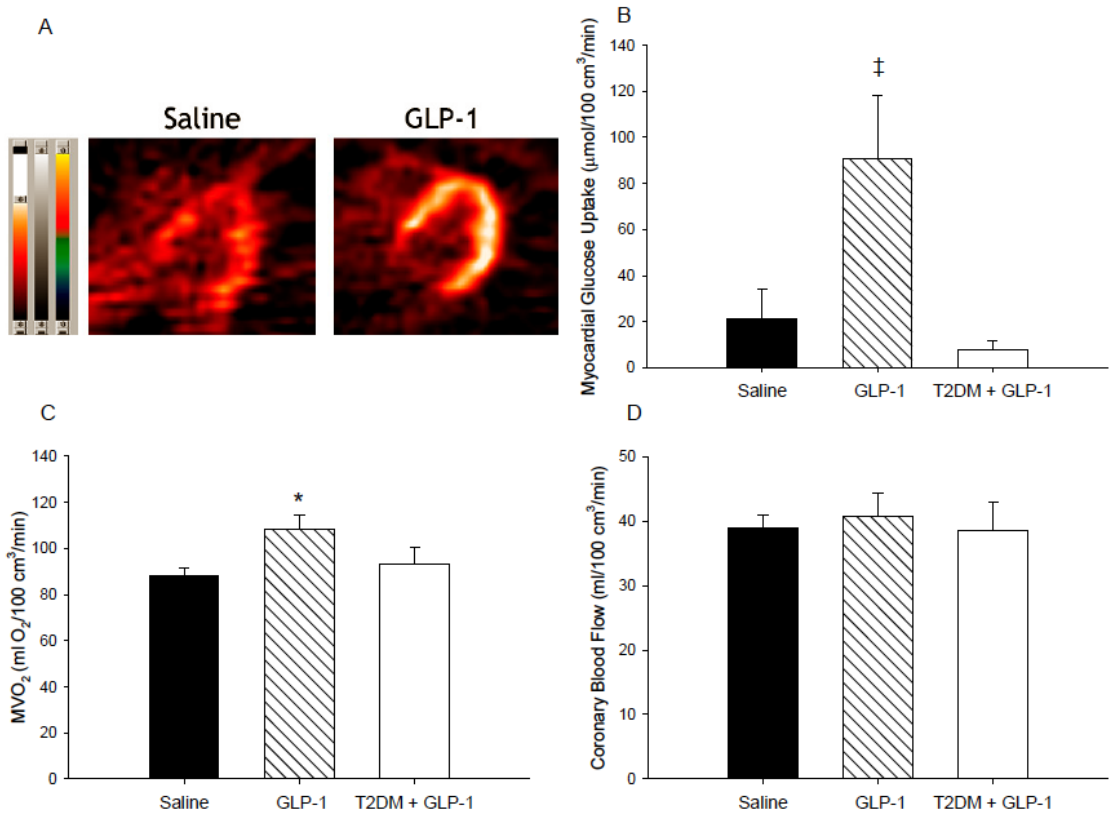

Figure 3-1 ***Effect of GLP-1 on myocardial glucose uptake, total oxidative metabolism, and blood flow in human subjects.*** A representative PET image for the effect of GLP-1 on myocardial glucose uptake in lean subjects **(A).** T2DM subjects treated with GLP-1 had myocardial glucose uptake lower than that of lean subjects treated with GLP-1, and not different than lean subjects given saline **(B)**. Myocardial Oxygen Consumption (MVO_2) was modestly elevated in lean subjects treated with GLP-1, but not different between lean saline control and MetS/T2DM + GLP-1 **(C)**. Coronary blood flow was not different between any groups. GLP-1 increased myocardial glucose uptake in lean subjects **(D)**. (‡) $P \leq 0.05$ vs. lean saline and T2DM +GLP-1; (*) $P \leq 0.05$ vs. lean saline

Phenotypic data in lean and MetS Ossabaw swine. In comparison to lean swine, high-fat fed swine had significantly elevated body weight, mean aortic pressure, triglycerides, cholesterol and LDL/HDL ratio. There were no differences between lean and MetS swine in fasting insulin, plasma glucose, or HOMA-IR; however, MetS swine did have hyperglucagonemia. (**Table 3-2A**)**.** GLP-1 had no

effect on the phenotypic plasma profiles of lean or MetS swine. Fasting levels of total GLP-1 and GLP-1 (7-36) were not different between lean and MetS swine, and the average plasma concentrations increased equally in both groups following GLP-1 (7-36) administration (1.5 pmol/kg/min, iv, 2 hrs) (**Table 3-2A**).

Table 3-2A *Phenotypic characteristics of lean and metabolic syndrome Ossabaw swine with and without GLP-1 (7-36) (1.5 pmol/kg/min).*

	Lean	Lean + GLP-1	MetS	MetS + GLP-1
Body Weight (kg)	46.6 ± 2.3		69.0 ± 2.5*	
Heart wt. / Body wt. (x 100)	0.37 ± 0.02		0.38 ± 0.01	
Glucose (mg/dl)	80 ± 5	75 ± 6	85 ± 5	75 ± 9
Insulin (μU/ml)	21 ± 5	20 ± 3	23 ± 3	18 ± 4
HOMA-IR	4.2 ± 1.0	3.7 ± 0.9	5.0 ± 0.9	3.6 ± 1.3
Glucagon (pg/ml)	103 ± 9	92 ± 12	146 ± 22*	160 ± 19*
Total cholesterol (mg/dl)	89 ± 5	100 ± 6	473 ± 92*	470 ± 93*
LDL/HDL ratio	1.6 ± 0.1	1.7 ± 0.1	6.5 ± 1.9*	4.3 ± 0.4*
Triglycerides (mg/dl)	45 ± 5	40 ± 5	71 ± 13*	83 ± 13*
Lactate (mg/dL)	1.1 ± 0.1	1.0 ± 0.4	2.3 ± 0.7*	1.8 ± 0.5
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 MetS (n = 5) swine. * $P \leq 0.05$ vs lean same condition.

Table 3-2B *Hemodynamic variables at rest and during graded treadmill exercise in lean and metabolic syndrome Ossabaw swine with and without GLP-1 (7-36) (1.5 pmol/kg/min).*

	Exercise Level		
	Rest	**Level 1**	**Level 2**
Systolic Blood Pressure (mmHg)			
Lean	111 ± 3	118 ± 3	119 ± 9
Lean + GLP-1	117 ± 8	118 ± 7	126 ± 6
MetS	128 ± 7*	125 ± 4	134 ± 5
MetS + GLP-1	114 ± 7	125 ± 9	144 ± 9†
Diastolic Blood Pressure (mmHg)			
Lean	73 ± 3	79 ± 2	80 ± 3
Lean + GLP-1	74 ± 5	77 ± 4	83 ± 5
MetS	87 ± 7	80 ± 5	86 ± 4
MetS + GLP-1	86 ± 5	87 ± 5	100 ± 6
Mean Aortic Pressure (mmHg)			
Lean	94 ± 2	100 ± 2	106 ± 2†
Lean + GLP-1	99 ± 7	99 ± 6	105 ± 5
MetS	108 ± 7*	102 ± 4	110 ± 4
MetS + GLP-1	100 ± 6	106 ± 7	122 ± 7
Heart Rate (beats/min)			
Lean	147 ± 11	188 ± 9†	221 ± 9†‡
Lean + GLP-1	148 ± 8	187 ± 10†	220 ± 8†‡
MetS	134 ± 9	176 ± 13†	192 ± 18†
MetS + GLP-1	137 ± 12	182 ± 14†	197 ± 16†
Myocardial O_2 Consumption (μl O_2/min/g)			
Lean	119 ± 14	185 ± 22†	218 ± 18†
Lean + GLP-1	103 ± 14	172 ± 22†	190 ± 25†
MetS	113 ± 8	174 ± 25†	184 ± 27†
MetS + GLP-1	110 ± 18	155 ± 21	196 ± 28†
Myocardial Blood Flow (ml/min/g)			
Lean	1.0 ± 0.1	1.5 ± 0.2†	1.6 ± 0.1†
Lean + GLP-1	1.3 ± 0.2	1.6 ± 0.2	1.8 ± 0.2
MetS	1.0 ± 0.1	1.3 ± 0.2	1.4 ± 0.2
MetS + GLP-1	1.0 ± 0.1	1.2 ± 0.2	1.5 ± 0.2

Values are mean ± SE for lean (n = 7) and MetS (n = 5) swine. * $P \leq 0.05$ vs lean same condition, † $P \leq 0.05$ vs rest same group, ‡ $P \leq 0.05$ vs level 1 same group

Hemodynamic effects of GLP-1 (7-36) in resting and exercising swine.

Resting systolic and mean aortic blood pressure were significantly elevated in

MetS swine compared to lean swine, and diastolic blood pressure tended to be higher in MetS swine (P = 0.057). There was no difference in resting heart rate between lean and MetS swine (**Table 3-2B**). Administration of GLP-1 had no effect on blood pressure or heart rate at rest or in response to exercise in lean or MetS swine**.** Furthermore, coronary blood flow and MVO_2 were not different between lean and MetS swine at rest or during exercise, and GLP-1 had no significant effect on these measures in either group (**Table 3-2B**)**.**

Effects of GLP-1 (7-36) on myocardial substrate uptake in lean and MetS Swine. In lean swine GLP-1 stimulated a significant increase in the slope of the relationship between increasing MVO_2 (independent variable) and myocardial glucose uptake during exercise (**Figure 3-2A**)**.** In MetS swine, there was a paradoxical decrease in the slope of the relationship between myocardial glucose uptake and MVO_2 (**Figure 3-2B**)**.** Resting arterial lactate was significantly elevated in MetS swine compared with lean swine (**Table 3-2A**), and lactate uptake increased as a function of MVO_2 in both lean and MetS swine; GLP-1 had no effect on this relationship (**Figures 3-2C and 3-2D**)**.**

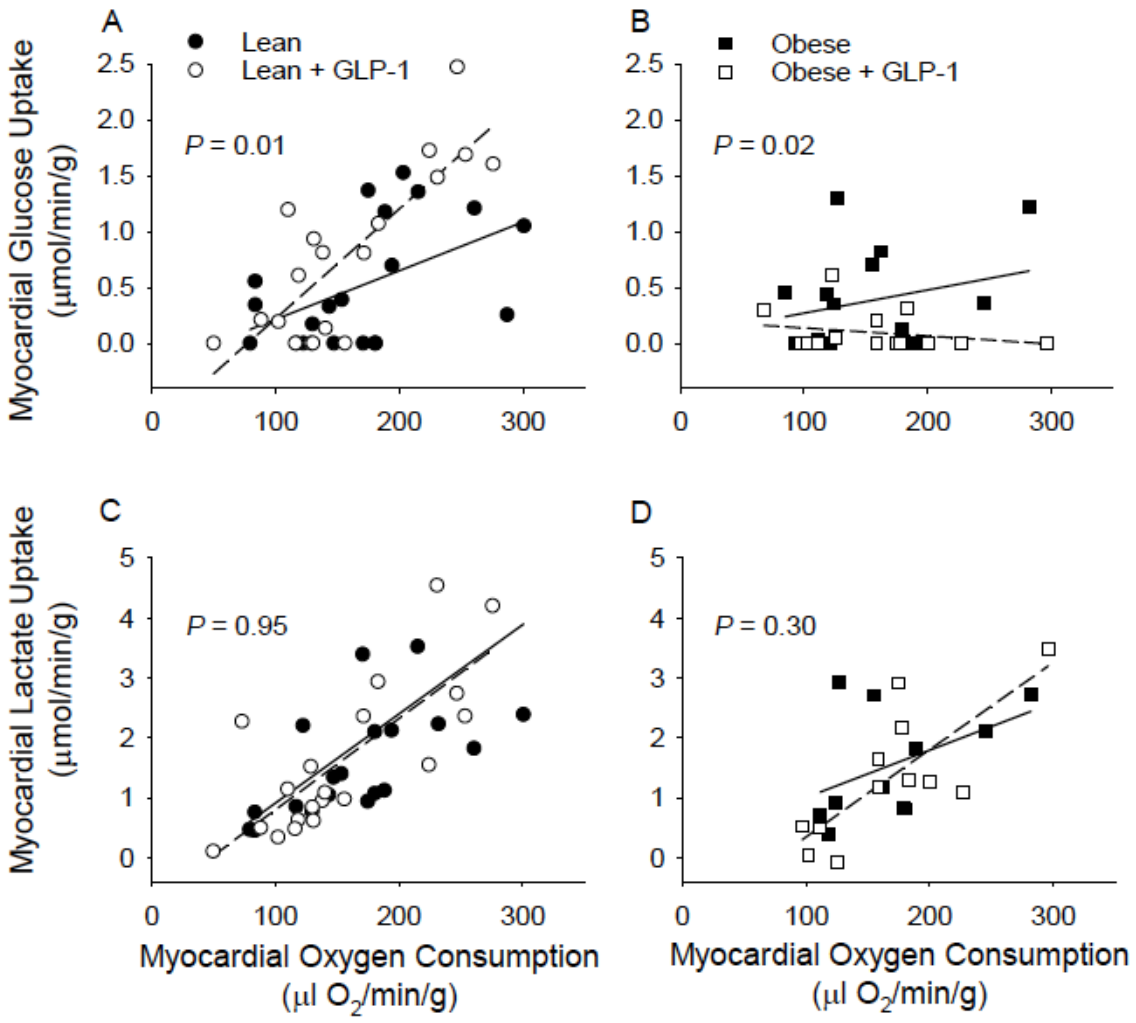

Figure 3-2 ***Effects of GLP-1 on myocardial substrate metabolism in exercising Ossabaw swine.*** GLP-1 (1.5 pmol/kg/min iv, 2 hrs) increased myocardial glucose uptake in response to increasing myocardial oxygen consumption in exercising lean **(A)** but not MetS **(B)** swine. Myocardial lactate uptake was not affected by GLP-1 in either lean **(C)** or MetS **(D)** swine.

Myocardial and coronary expression of GLP-1R. Immunohistochemistry with confocal fluorescence microscopy revealed both myocardial and trans-adventitial coronary artery expression of GLP-1R in Ossabaw swine (**Figure 3-3A**). Counterstaining the tissue with 4',6-diamidino-2-phenylindole (DAPI) and fluorescent-linked antibodies raised against cardiac troponin I demonstrate the

presence of nuclei (blue) and myocardium (red) respectively, adding detail to the tissue architecture (**Figure 3-3B**). A representative western blot qualitatively demonstrates no difference in cardiac GLP-1R content between lean and MetS swine, and depicts clean immunoreactivity at the expected size band for GLP-1R at ~53 kDa. The internal control α-actin is demonstrated as a band at ~42 kDa (**Figure 3-3C**). Quantification of western blots revealed no differences between lean and MetS swine in the total coronary or crude cardiac GLP-1R content (**Figure 3-3D**).

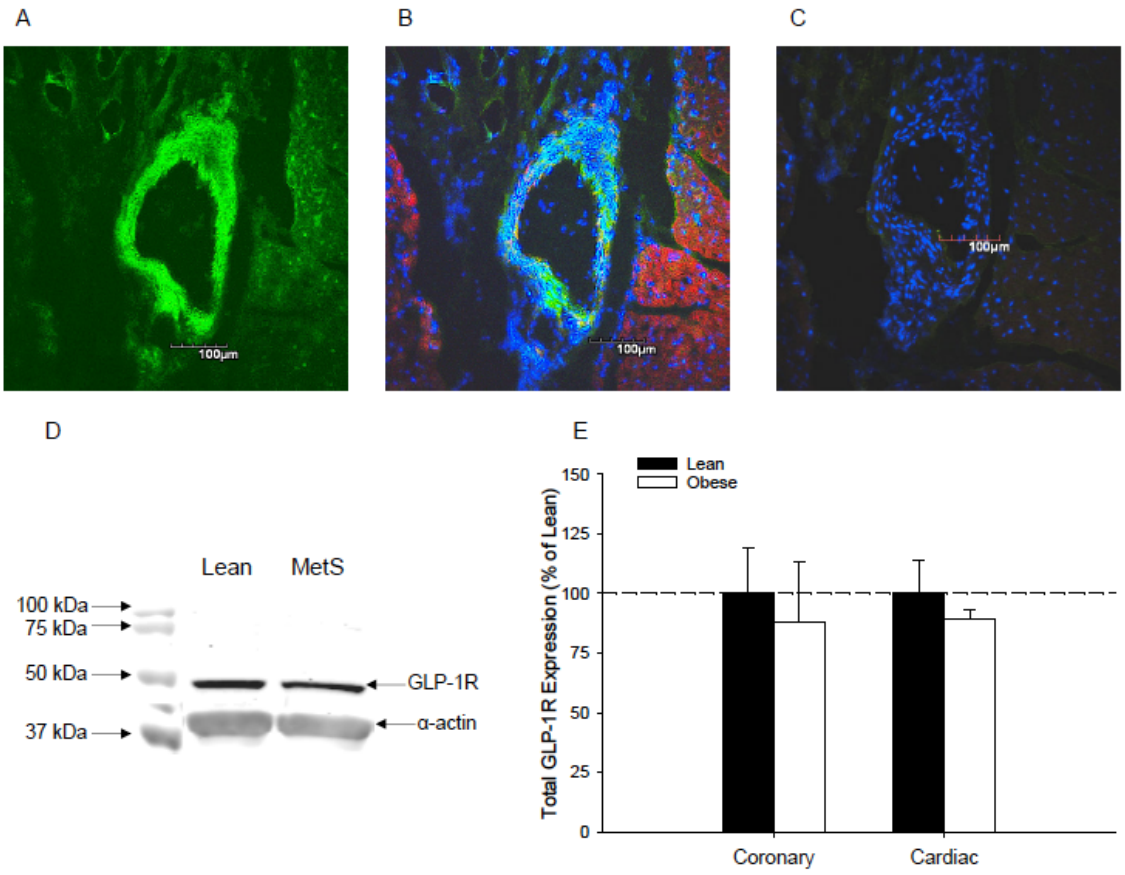

Figure 3-3 ***Cardiac GLP-1R expression in Ossabaw swine.*** GLP-1R **(green)** was present in the myocardium and coronary microvessels of Ossabaw swine **(A)**. Tissue architecture is further demonstrated **(B)** with the nuclear stain DAPI **(blue)** and antibodies against cardiac troponin I **(red)**. A negative control depicts low tisuue auto fluorescence **(C)**. Western Blot revealed the expected molecular weight bands for GLP-1R (~53 kDa) and the loading control alpha actin (~42 kDa) in cardiac tissue from lean and MetS swine **(D)**. There were no differences between lean and MetS swine in either coronary or crude cardiac GLP-1R expression **(E)**.

Effects of GLP-1 on cardiac PKA and p-38 MAPK enzyme activity. Basal and cAMP stimulated PKA activity differed as expected in untreated (sham control) cardiac tissue from lean swine (0.054 Abs_{492} at baseline vs. 1.367 Abs_{492} with cAMP); however, there were no differences in basal or cAMP stimulated PKA activity between lean and MetS groups. Treatment of fresh cardiac tissue

slices with GLP-1 (7-36) (1 to 5 nmol/L) for 1 hour in a tissue bath of physiologic salt solution at 37˚C did not affect basal or cAMP stimulated PKA activity when compared to untreated sham controls in tissue from either lean or MetS swine (**Figure 3-4**).

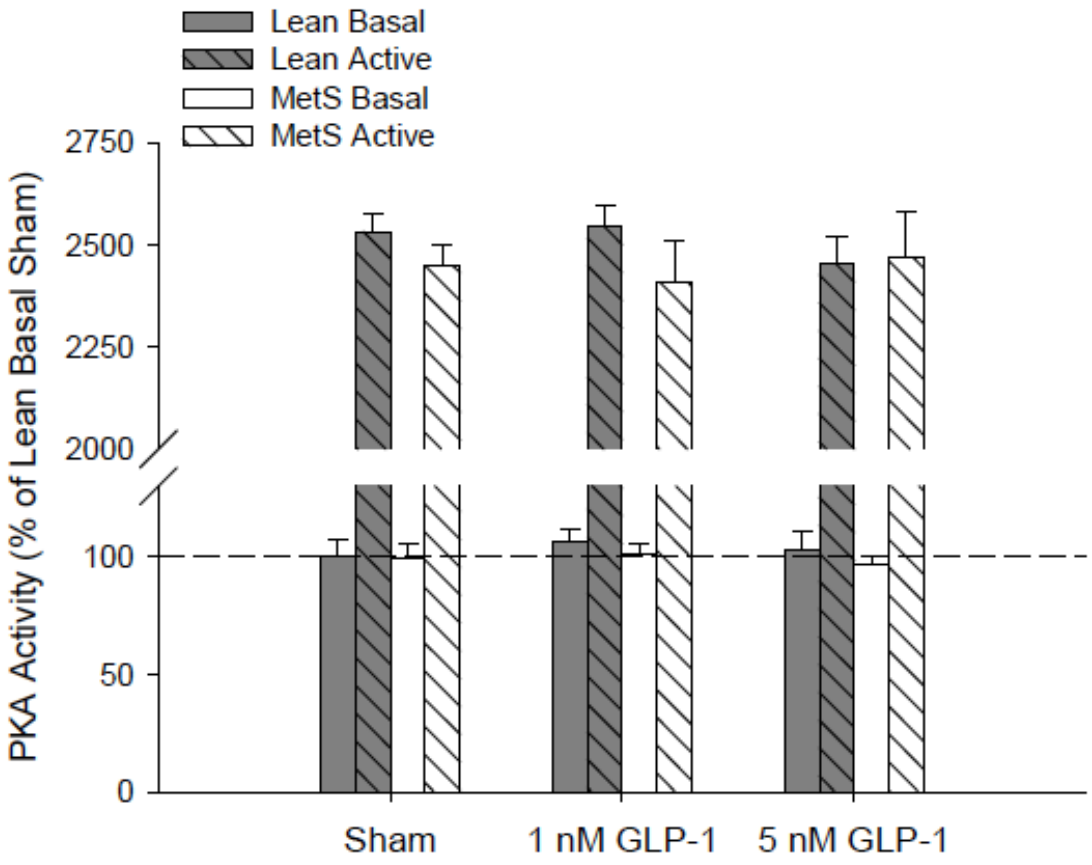

Figure 3-4 ***Effect of GLP-1 on cardiac PKA activity in Ossabaw swine.*** Treatment of cardiac slices with GLP-1 (1 nmol/L to 5 nmol/L) for 1 hr had no effect on basal PKA activity in tissue from lean and MetS swine. Addition of the PKA activator cAMP to the reaction mixture did not affect the relative activity between GLP-1 treated and untreated tissue from lean or MetS swine.

A representative western blot demonstrates selective immunoreactivity and abundant expression of p38α-MAPK at the expected band size of ~41 kDa. The band for the internal control α-actin (~42 kDa) appears just above that of

p38α-MAPK (**Figure 3-5A**). Total p38α-MAPK content was not different in cardiac tissue from lean or MetS swine (**Figure 3-5B**). Enzymatic activity of p38-MAPK in cardiac tissue from lean and MetS swine treated with saline (sham) or GLP-1 (7-36) is demonstrated with immunoreactivity to the p38-MAPK product phosopho-ATF-2 (~34 kDa) (**Figure 3-5C**). MAPK activity dose-dependently increased in response to GLP-1 administration in cardiac tissue from lean swine. In contrast, p38-MAPK activity was markedly diminished in hearts from MetS swine under control conditions and was unchanged by GLP-1 administration (**Figure 3-5D**).

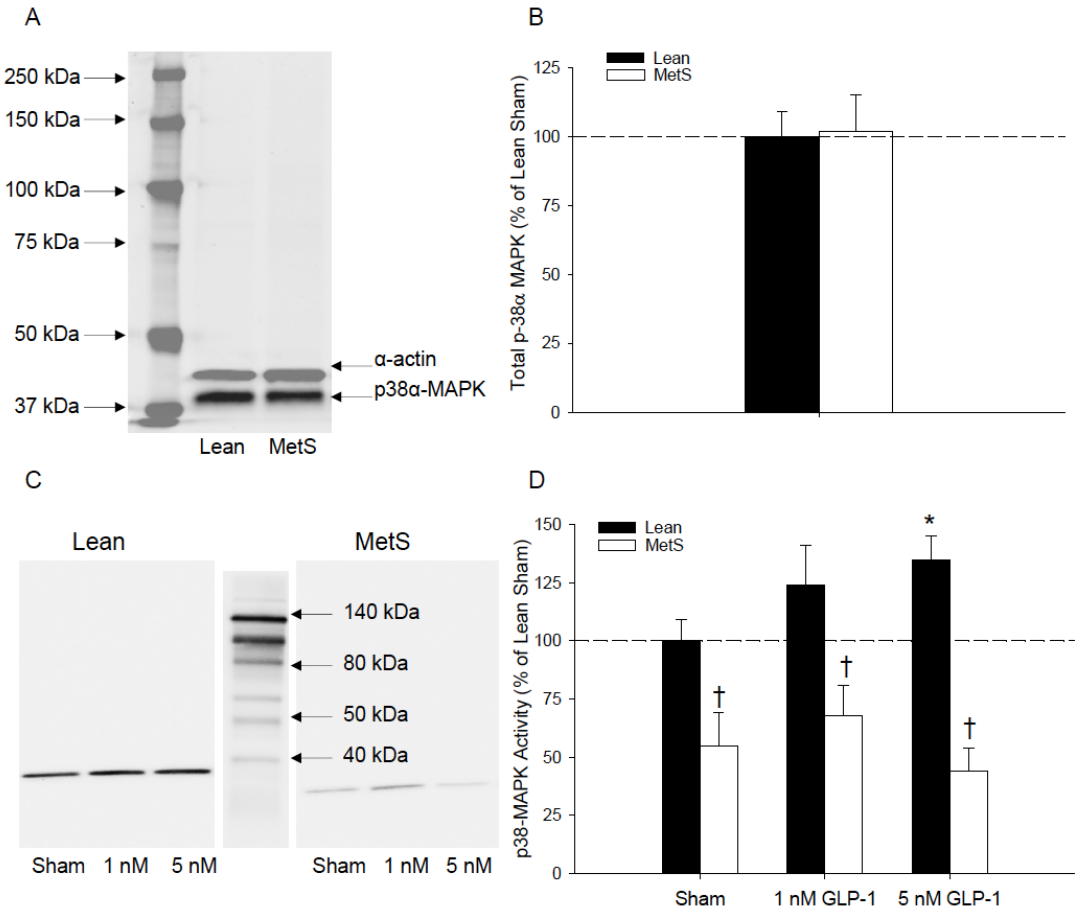

Figure 3-5 ***Effect of GLP-1 on cardiac p38-MAPK activity in Ossabaw swine.*** A representative image of total cardiac p38α-MAPK from lean and MetS swine **(A)**. There was no difference in total cardiac expression of p38α-MAPK between lean and MetS swine **(B)**. A representative image from the enzyme activity assay demonstrates differential presence of the p38-MAPK product Phospho-ATF-2 (~ 34 kDa) **(C)**.Treatment of cardiac slices with GLP-1 (1nmol/L to 5 nmol/L) for 1 hr increased p38-MAPK activity in tissue from lean but not MetS swine, and activity was lower in tissue from MetS swine at all levels of treatment **(D)**. (*) $P \leq 0.05$ vs. lean sham; (†) $P \leq 0.05$ vs. lean same condition

Discussion

This investigation was designed to examine the cardiovascular effects of GLP-1 in the setting of the MetS and T2DM. The major novel findings from these translational studies in T2DM humans and obese swine were: 1) GLP-1 administration had no effect on blood pressure, heart rate, or coronary blood flow

at rest or during exercise-induced increases in MVO_2 in lean or obese/T2DM subjects; 2) GLP-1-mediated increases in myocardial glucose uptake are significantly diminished in obesity/MetS and T2DM; 3) impaired myocardial responsiveness to GLP-1 is not associated with alterations in GLP1R expression or activation of cAMP/PKA signaling; 5) GLP-1 dose-dependently activates p38-MAPK in normal-lean, but not obese/MetS hearts. Taken together, these findings indicate that the potential cardioprotective effects of GLP-1 on myocardial glucose metabolism are attenuated in obesity and T2DM via reductions in p38-MAPK activity.

Effects of GLP-1 on systemic hemodynamics, cardiac function, and coronary blood flow. Heart rate, blood pressure, cardiac output, and stroke volume were not affected by GLP-1 administration in either lean or MetS/T2DM human subjects **(Table 3-1B)**. In swine, heart rate and blood pressure were also unaffected by GLP-1 at rest and during exercise **(Table 3-2B)**. Numerous investigations have demonstrated that iv GLP-1 increases cardiac output, stroke volume, and other parameters of left ventricular performance in the settings of ischemia-reperfusion injury and/or heart failure (87-90, 94, 98, 110). However, our present findings indicate that GLP-1 does not affect systemic hemodynamics or left ventricular performance in the non-injured/non-failing heart.

We also found that systemic administration of GLP-1 had no effect on coronary blood flow in lean vs. T2DM humans **(Figure 3-1D),** or in lean vs. obese/MetS swine **(Table 3-2B)**. Previous data regarding the effects of GLP-1 on coronary blood flow have been equivocal, as intracoronary GLP-1 (7-36) has

been reported to increase perfusion in isolated rodent hearts (91, 93) but have no effect *in-vivo* in canines (185). Most studies examining the effects of intravenous GLP-1 on coronary blood flow in canines and swine also report no vasodilator effect (94, 110, 133). However, one study that reported a modest yet significant increase in coronary flow also found that GLP-1 increased MVO_2; i.e. increase is coronary blood flow was likely the result of metabolic dilation, not vasodilation in response to GLP-1 (89). These findings are consistent with previous data from our laboratory, which showed no effect of GLP-1 (10 pmol/L to 1 nmol/L) on isolated intact or endothelium denuded coronary arteries (185).

Effects of GLP-1 on myocardial substrate metabolism in MetS/T2DM. The major findings of this study demonstrate that the cardiac action of iv GLP-1 to increase myocardial glucose uptake is significantly attenuated in obesity/MetS and T2DM (**Figures 3-1A, 3-1B, 3-2A and 3-2B**). This impaired cardiometabolic response to GLP-1 is clinically significant because augmented myocardial glucose uptake is indicated as an important mechanism by which GLP-1 protects the heart from injury/infarction (53, 89, 91, 93, 110). Importantly, this attenuated response in MetS-T2DM is not related to differences in plasma GLP-1 concentration (**Tables 3-1A and 3-2A**). Furthermore, similar plasma concentrations of glucose and NEFA in humans also indicate that the impaired response to GLP-1 is not related to alterations in plasma substrate availability (**Table 3-1A**). Although this study indicates no effect of GLP-1 on myocardial lactate uptake (**Figure 3-2C and 3-2D**), alterations in other substrates (i.e. free fatty acids) is needed; elevated myocardial glucose uptake in lean subjects vs.

MetS/T2DM with a post-treatment MVO_2 that is not different (**Figure 3-1C and Table 3-2B**), suggest iv GLP-1 decreases cardiac free fatty acid utilization in lean but not MetS/T2DM subjects.

Potential mechanisms for impaired cardiometabolic effects of GLP-1 in MetS/T2DM. To uncover potential mechanisms underlying the impaired cardiometabolic responses to GLP-1 in the setting of MetS and T2DM, we examined coronary and myocardial GLP-1R expression, as well as PKA and p38-MAPK activity. Our immunohistochemistry studies confirm GLP-1R expression in both myocardium and the coronary vasculature (**Figures 3-3A and 3-3B**). Importantly, Western blot analysis revealed no difference in coronary or myocardial GLP-1R content between lean and MetS swine, suggesting the impaired responses to GLP-1 in MetS are not mediated by a decrease in total GLP-1R expression (**Figures 3-3C and 3-3D**).

Previous investigations indicate that the myocardial effects of GLP-1 are mediated via activation of cAMP/PKA and/or p38-MAPK signaling pathways (91, 93, 94, 101, 182). To examine potential effects on each of these pathways, we performed studies on myocardial tissue slices exposed to either sham-vehicle or GLP-1 (1 nmol/L or 5 nmol/L). We found no differences in basal or cAMP stimulated cardiac PKA activity between lean and MetS swine, and treatment with GLP-1 had no effect on PKA activity in either group (**Figures 3-4**). These data are consistent with the lack of effect of GLP-1 to increase cardiac contractile function in normal hearts (**Table 3-1B**; (91, 185)) and also argues against a role for GLP-1 in activating classical inotropic (β-adrenergic) signaling pathways. In

contrast however, we did find that GLP-1 dose-dependently increased p38-MAPK activity in lean-control hearts (**Figure 3-5C and 3-5D**). Importantly, no differences in the overall level p38α-MAPK expression were detected in lean vs. MetS swine hearts; i.e. total cardiac p38-MAPK expression levels were not affected by MetS (**Figure 3-5A and 3-5B**). Interestingly, basal p38-MAPK activity was significantly lower in cardiac tissue from MetS swine, and did not respond to treatment with GLP-1 (**Figures 3-5C and 3-5D**). These data indicate that the cardiometablic effects of GLP-1 are mediated by a non-classical GLP-1 signaling pathway involving p38-MAPK, and not cAMP/PKA. This finding is supported by previous investigations in canines demonstrating that p38-MAPK activity is increased in response to GLP-1 treatment, and that inhibition of p38-MAPK abolished the effect of GLP-1 to increase myocardial glucose uptake.

Conclusion

Data from this investigation provide novel evidence that the myocardial effect of GLP-1 to increase glucose uptake is impaired in the settings of MetS and T2DM in association with defects in p38-MAPK signaling. These findings are significant because drug therapies used to increase myocardial glucose uptake have been targeted as potential treatment and management of heart disease for decades (52, 57, 112, 113, 116, 118, 122, 186, 187). Previous investigations have demonstrated that GLP-1 based therapies are useful in protection from and treatment of cardiac injury/failure (87-94, 97, 98, 100-102, 104-107, 140, 180, 188-190), and increases in glucose metabolism are believed to play a key role in these clinical outcomes (89, 91, 93, 94, 110, 185). Currently GLP-1 based

therapies are widely used for glycemic control in patients with T2DM (179, 191, 192), and our results indicate that the effects of GLP-1 to reduce cardiac damage and increase left ventricular performance in the settings of ischemia/reperfusion injury and heart failure may be impaired by MetS and T2DM. However, future studies are needed to examine if the cardiometabolic deficit translates into a cardioprotective deficit. If the defect in glucose response equates to a defect in cardioprotective actions, strategies to overcome this impaired action and improve the beneficial cardiac effects of GLP-1 to patients with MetS/DM will be needed.

Acknowledgements

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Chapter 4

Discussion

As the prevalence of obesity, MetS and T2DM continue to rise, there is an imminent health-care need to develop new therapeutic strategies to address the heightened incidence of cardiovascular morbidity and mortality in these patient populations (1, 4, 10, 147-149). While reasons for an increased incidence of adverse cardiac events and outcomes in these populations are still active areas of investigation, coronary microvascular dysfunction and diminished cardiac glucose metabolism have been implicated as common underlying morbidities (11, 13, 14, 17-20, 22, 26, 27, 29, 33, 40, 43, 53-56). Thus, improving myocardial oxygen delivery and promoting more efficient cardiac substrate utilization; i.e. restoring the balance of myocardial oxygen supply and demand, are primary targets for advancing the treatment and management of obesity-related cardiovascular disease (14, 20, 26, 53, 150-152).

Recent advancements in the management of systemic glucose regulation in obesity/T2DM include drug therapies designed to utilize components of the incretin system specifically related to glucagon-like peptide 1 (GLP-1) (169, 173, 174, 193-195). More recently, GLP-1 has been investigated for potential cardioprotective effects. Several investigations have revealed that intravenous administration of GLP-1 significantly reduces myocardial infarct size following ischemia/reperfusion injury and improves cardiac contractile function in the settings of coronary artery disease, myocardial ischemia/reperfusion injury, and heart failure (87-90, 94, 98, 110, 180, 181). While the mechanisms responsible for these effects of GLP-1 are still under investigation, increases in myocardial

glucose uptake and coronary blood flow via a p38 MAPK and/or PKA dependent signaling pathway have been implicated (91, 93, 94, 101, 182).

Despite an abundance of data indicating that intravenous infusion of GLP-1 is cardioprotective, information has been lacking on the cardiac effects of iv GLP-1 in the MetS or T2DM population. It was recently recognized that the insulinotropic effect of GLP-1 is diminished, although still effective, in the setting of obesity/T2DM (95). However, whether obesity, MetS, and/or T2DM alter the cardiac actions of GLP-1 has not been directly evaluated. Thus, while GLP-1 may be useful in the treatment and management of obesity related heart disease by improving coronary microvascular function and myocardial glucose uptake, there is a distinct possibility that the cardiac actions of GLP-1 are impaired in the settings of obesity/MetS and T2DM.

Some important questions this study aimed to address are 1) what are the direct, dose-dependent cardiac effects of GLP-1 *in-vivo* 2) are the cardiac effects influenced by cardiac demand (MVO_2) and/or ischemia, 3) does GLP-1 effect myocardial blood flow, glucose uptake or total oxidative metabolism in human subjects, and 4) are the cardiac effects of GLP-1 treatment impaired in the settings of obesity/MetS and T2DM.

In an effort to address these questions we conducted translational studies in open-chest canines, our novel Ossabaw swine model of obesity/MetS, human subjects with MetS/T2DM, as well as lean swine and human subjects. The major objectives of this study were to more fully elucidate the cardiac actions of GLP-1, determine if these actions are impaired in the setting of obesity/MetS, and

uncover potential mechanisms of impairment. These objectives were met by pursuing the following Specific Aims:

Aim 1: ***Determine the acute, dose-dependent cardiac effects of intracoronary GLP-1 under conditions of normal coronary perfusion and during ischemia.*** Initial studies conducted in canines demonstrated that GLP-1 had no direct effect on coronary blood flow *in-vivo* or vasomotor tone *in-vitro*, but preferentially increased myocardial glucose uptake in ischemic myocardium independent of effects on cardiac contractile function or coronary blood flow.

Aim 2: ***Test the hypothesis that obesity/MetS impairs the cardiac responses to GLP-1, and investigate potential mechanisms of such impairment.*** Parallel translational studies conducted in the humans and Ossabaw swine demonstrate that iv GLP-1 significantly increases myocardial glucose uptake at rest and in response to increases in cardiac demand (MVO_2) in lean subjects, but not in the settings of obesity/MetS and T2DM. Further investigation in isolated cardiac tissue from lean and obese/MetS swine indicate that this impairment in GLP-1 responsiveness is related to attenuated activation of p38-MAPK, independent of alterations in GLP-1 receptor expression or PKA-dependent signaling.

Implications

We have produced the first evidence for impaired cardiometabolic responses to GLP-1 in obesity, MetS and T2DM (**Figures 3-1A, 3-1B, 3-2A and 3-2B**). Results from this study indicate that the impairment in GLP-1 responsiveness is related to attenuated activation of p38-MAPK (**Figure 3-5C**

and 3-5D), and is independent of alterations in coronary blood flow (**Figure 3-1D and Table 3-2B**), GLP-1 receptor expression (**Figure 3-3**), and PKA-dependent signaling (**Figure 3-4**).

Coronary vascular effects of GLP-1. Our findings demonstrate that although GLP-1R is expressed in the coronary vasculature (**Figures 2-1 and 3-3**), administration of GLP-1 does not increase coronary blood flow in canines (**Figure 2-2**), swine (**Table 3-2B**) or humans (**Figure 3-1B**). These data are in contrast with earlier studies which documented that GLP-1 induces vasodilation in isolated arteries, increases coronary blood flow in isolated hearts under normal conditions and during reperfusion, and following pacing induced cardiomyopathy in canines. However, such previous studies were conducted in aorta (130, 132), femoral (129), and pulmonary vessels (131), isolated rodent hearts (91, 93), and with systemic administration of recombinant GLP-1 (7-36) in canines (89). It is also important to recognize that the increased coronary blood flow previously documented *in-vivo* in canines was accompanied by increases in cardiac contractile function and MVO_2, the primary determinants of coronary flow; i.e. metabolic vasodilation. Other studies conducted in this same canine model, and by the same laboratory, demonstrated no effect of GLP-1 on MVO2 or coronary blood flow (94, 110). The reasons for the discrepant findings regarding the vascular effects of GLP-1 are unclear but are likely related to differences in specific vessels studied, species investigated, *in-vivo* vs. *in-vivo* experimental conditions. Regardless, our findings provide strong evidence that neither acute administration of physiologic/pharmacologic concentrations of GLP-1 directly in

to the coronary circulation, nor longer-term iv GLP-1 administration influence coronary blood flow.

Effects of GLP-1 on hemodynamics and cardiac function. This investigation revealed no effect of iv GLP-1 on heart rate, blood pressure, stroke volume or cardiac output in lean or MetS/T2DM human subjects at rest (**Tables 3-1B and 3-2B**). We also observed no effect of iv GLP-1 on heart rate or blood pressure during exercise induced increases in cardiac demand (**Table 3-2B**). Several previous investigations have reported that GLP-1 increases indices of LV function (i.e. developed pressure, wall thickening, dP/dt and ejection fraction) during reperfusion, heart failure and dilated cardiomyopathy (87-92, 94, 98, 99, 101-103, 110, 140, 180, 181). However, our present findings indicate that GLP-1 does not effect systemic hemodynamics or left ventricular performance in the non-injured/non-failing heart.

We also observed no direct effect of acute intracoronary GLP-1 (7-36) infusion on indices of regional or global cardiac contractile function during ischemia (**Table 2-1 and Figure 2-4**). This finding is in contrast to our hypothesis that increases in glucose metabolism would improve contractile function in ischemic myocardium, as glucose has a high P/O ratio (ATP produced per oxygen consumed, (53, 118) and earlier data support that increased myocardial glucose metabolism improves cardiac efficiency during ischemia. In particular, previous studies from our group documented that insulin-mediated increases in myocardial glucose metabolism significantly improve regional cardiac function in ischemic canine hearts (CPP = 40 mmHg) (52, 57). However, the increase in

myocardial glucose uptake induced by insulin in these studies (~0.50 mmol/min/g) was substantially higher than that reported in the present study (~0.20 μmol/min/g). A longer infusion duration of GLP-1 (500 to 1000 pmol) may be necessary to obtain greater cardiac glucose uptake and improved left ventricular function.

Interestingly, we did observe a moderate dose-dependent decrease in stroke volume and cardiac output at normal perfusion pressure (CPP = 100 mmHg), without corresponding changes in dP/dt or aortic pressure (**Table 2-1**). This reduction in cardiac output was associated with an ~ 10 beats/min increase in heart rate. Although there were no changes in dP/dt in our experiments, the reductions in stroke volume and cardiac output suggest GLP-1 reduced contractile performance. This observation is supported by a previous study in which recombinant GLP-1 (7-36) decreased LV developed pressure and dP/dt when infused into the coronary circulation of isolated rat hearts (91). Taken together, these findings indicate that acute direct (intracoronary) effects of GLP-1 on cardiac function in undamaged hearts differ from indirect (peripheral/central) effects, or effects observed in the settings of heart failure, ischemia, and reperfusion injury.

Cardiometabolic effects of GLP-1. The major findings of this study demonstrate that several hours of iv GLP-1 markedly increase myocardial glucose uptake in resting lean human subjects (**Figure 3-1A and 3-1B**), and that shorter durations of iv administration and acute intracoronary administration are sufficient to increase myocardial glucose uptake under conditions of cardiac stress (i.e.

increased cardiac demand (MVO2) and ischemia, **Figures 3-2 and 2-5**). This finding is consistent with previous data indicating that GLP-1 augments myocardial glucose uptake under conditions of cardiac stress (e.g. ischemia/reperfusion injury, dilated cardiomyopathy) (89, 91, 110). Also consistent with previous investigations, the current study revealed little to no effect of GLP-1 on MVO_2 at rest (89, 94, 110), and extending this, there was no effect on MVO_2 during ischemia or exercise (**Figures 2-5A, 3-1C, and Table 3-2B**). Importantly, data from this investigation are the first to demonstrate that myocardial glucose uptake was not augmented with GLP-1 in the conditions of obesity/MetS or T2DM relative to lean and saline control groups (**Figures 3-1 and 3-2**). Furthermore, this investigation demonstrated dose dependence for the cardiac insulinomemetic effect of GLP-1 in the open-chest canine experiments (**Figure 2-5**). Our results indicate that systemic administration of GLP-1 is not mandatory for the insulinomimetic effect to be manifest and argue against peripheral/central actions of GLP-1 driving alterations in substrate metabolism. Studies conducted in the Shannon laboratory indicate that the effects of GLP-1 on myocardial glucose uptake occuring with several hours of iv exposure are mediated by GLP-1 (9-36), are dependent on p38-MAPK signaling, and occur in the absence of alterations in adenylate cyclase activity (94, 110). However, whether the acute effects of GLP-1 (7-36) on ischemic myocardial glucose metabolism are dependent on GLP-1R activation and/or actions of the (9-36) degradation product merits further investigation. A proposed mechanism by which GLP-1 increases myocardial glucose uptake is presented in **Figure 4-1**.

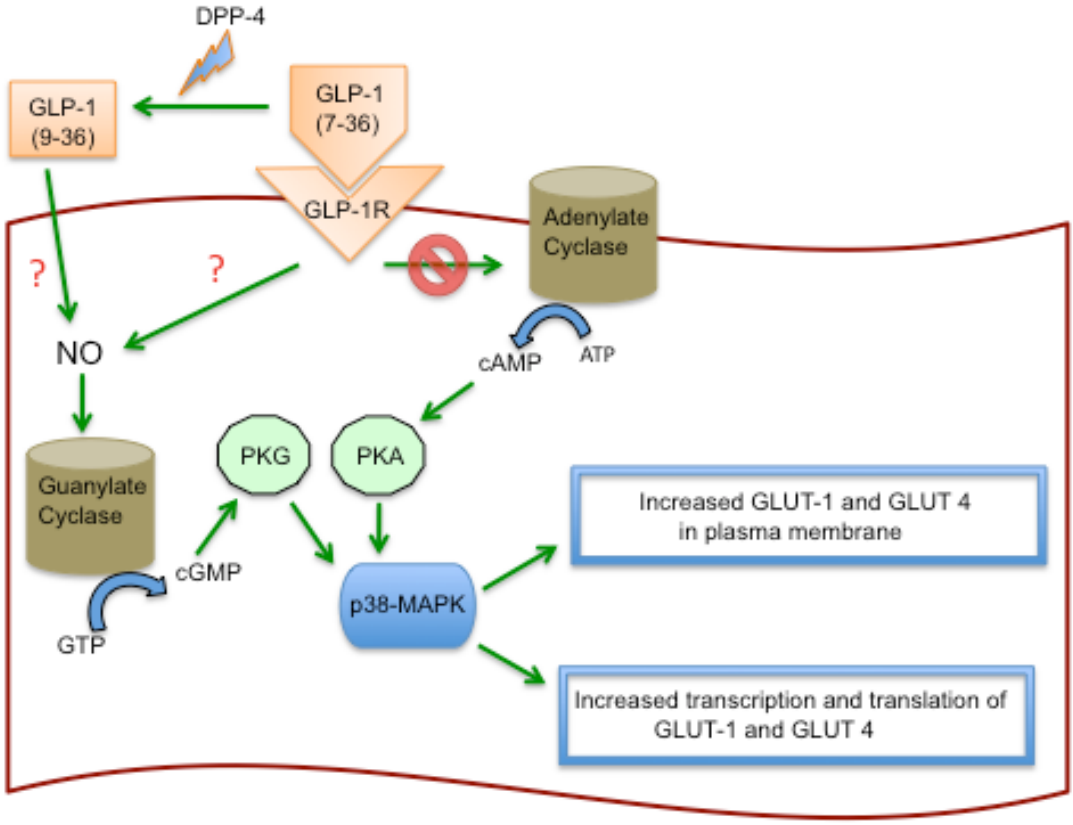

Figure 4-1 ***Proposed signaling mechanisms by which GLP-1 increases myocardial glucose uptake.*** GLP-1 can increase myocardial membrane content of both GLUT-1 and GLUT-4. Data indicate a mechanism dependent on NO and p38-MAPK, but independent of cAMP/PKA. GLP-1 (9-36) has been demonstrated to augment myocardial glucose uptake, indicating a GLP-1R independent pathway (91, 94, 110).

Clinical Implications and Future Direction

The impaired action of GLP-1 to increase myocardial glucose uptake in the settings of MetS and T2DM is clinically significant because drug therapies used to increase myocardial glucose uptake have been targeted as potential agents in the treatment and management of heart disease for decades (52, 57, 112, 113, 116, 118, 122, 186, 187). Previous investigations have demonstrated that GLP-1 based therapies are useful in improving cardiac function and reducing

infarct size in the settings of cardiac injury/failure (87-94, 97, 98, 100-102, 104-107, 140, 180, 188-190), and increases in glucose metabolism are believed to play a key role in these clinical outcomes (89, 91, 93, 94, 110, 185). Currently GLP-1 based therapies are widely used for glycemic control in patients with T2DM (196-198) and our results indicate that the effects of GLP-1 to reduce cardiac damage and increase left ventricular performance may be impaired by obesity/MetS and T2DM.

Future studies will be useful in further defining the effects of GLP-1 on cardiac substrate metabolism, and determining if the cardiometabolc deficit (impaired myocardial glucose uptake) observed in MetS/T2DM translates into a cardioprotective deficit. It still remains unclear if the changes in myocardial glucose uptake are accompanied by alterations in fatty acid utilization. Triple tracer PET studies to examine the effects of GLP-1 on myocardial glucose uptake, fatty acid utilization, and total oxidative metabolism in combination would be useful in further detailing shifts in cardiac substrate utilization. Such studies conducted in control settings and following ischemia/reperfusion in lean and obese MetS Ossabaw swine can be used to detail dynamic shifts in cardiac substrate metabolism resulting from ischemic damage, GLP-1 and obesity/MetS, as well as determining infarct size. This would also address the underlying question of whether or not impaired responses to the cardiac insulinomemetic effects GLP-1 are translated into a cardioprotective deficit, and if alterations in glucose uptake are associated with shifts in fatty acid utilization. **Figure 4-2**

schematically outlines a similar longitudinal study that we conducted in a separate investigation.

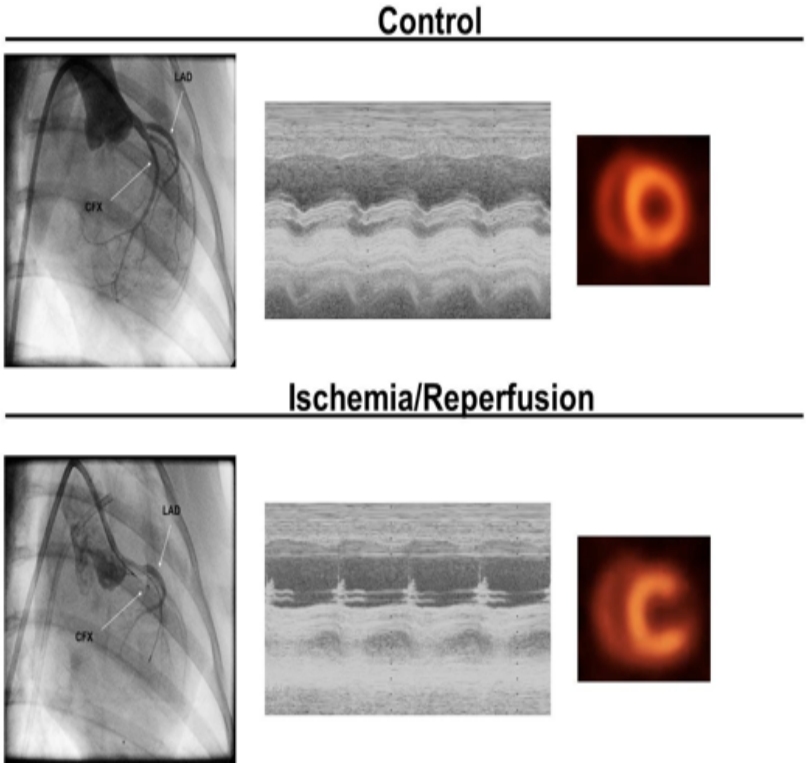

Figure 4-2 ***Imaging modalities demonstrating the feasibility of a longitudinal study on the cardiac effects of GLP-1.*** Left panels show angiography demonstrating coronary anatomy and experimental balloon occlusion of the left circumflex coronary artery (bottom left panel, 45 min occlusion). Center panels show M-mode transthoracic ultrasound demonstrating profound wall motion abnormalities following occlusion of the coronary artery. Right panels show ^{11}C-palmitate PET image demonstrating clear and readily quantifiable region of ischemia induced by focal occlusion (bottom right panel).

If the defect in glucose response equates to a defect in cardioprotective actions, strategies will need to be developed to overcome this impaired action and improve the beneficial cardiac effects of GLP-1 to patients with MetS/T2DM. However, this interdependence of effects has not been demonstrated and it is

possible that GLP-1 exerts protective effects distinct from those relating to glucose uptake.

Previous investigations have revealed that GLP-1 (9-36) has similar cardiac action as GLP-1 (7-36), thus indicating that the salutary cardiac actions of GLP-1 are in part mediated by GLP-1R independent mechanisms (110). The current investigation indicates that the conditions of obesity/MetS and T2DM render cardiometabolic resistance to both (or either) compound, as both total GLP-1 and GLP-1 (7-36) were elevated equally in lean vs. MetS-T2DM subjects following iv infusion of GLP-1 (7-36) (T**ables 3-1A and 3-2A**). However, further investigation into the cardiac actions of iv GLP-1 (9-36) in humans as well as large animal models should be pursued because common gastrointestinal side effects of GLP-1 are likely mediated by receptor dependent mechanisms. Longer iv infusion times of GLP-1 (7-36) allow the plasma concentration to become elevated to therapeutic levels gradually, and may decrease the incidence of nausea. Thus, the clinical implications for iv GLP-1 (9-36) may be superior to that of GLP-1 (7-36) as higher doses could potentially be administered, due to greater patient tolerance, and therapeutic plasma concentrations could be reached in a shorter time frame.

Further investigation into the mechanism by which GLP-1 increases myocardial glucose uptake is also warranted. The current investigation, in agreement with previous studies, demonstrated that intracoronary infusion of GLP-1 at concentrations of 500 to 1000 pmol acutely (within a few minutes) increases myocardial glucose uptake (91, 93, 185). There is evidence indicating

that GLP-1 can acutely increase myocardial glucose uptake by a mechanism involving GLUT-1 and not GLUT-4, and that this is mediated by nitric oxide and p38-MAPK (91, 94). There is also evidence that GLP-1 acutely increases both GLUT-1 and GLUT-4 in post-ischemic myocardium with the same cellular signaling events involving nitric oxide and p38-MAPK (91). Thus the effects of GLP-1 on GLUT-4 have previously been demonstrated to be conditional, and this is in agreement with the current study demonstrating that acute intracoronary GLP-1 preferentially increased myocardial glucose uptake in ischemic myocardium of canines (185). However, the effects of MetS and T2DM on GLP-1 mediated GLUT regulation has not been examined.

In order to examine the effects of GLP-1 on GLUT regulation in more detail, myocardial tissue slices from lean vs. MetS swine can be exposed to GLP-1 (7-36) or GLP-1 (9-36) without and with inhibitors of GLP-1R (exendin (9-39)) and p38-MAPK (SB202190). Sucrose gradient centrifugation can then be used to generate tissue fractionation and determine changes in GLUT-1/GLUT-4 distribution. These same techniques could be used to examine fatty acid transporters. Furthermore, possible defects in the ability of GLP-1 to trans-activate other pathways of intracellular fuel sensing and metabolic control can be examined in parallel, including AMPK, ACC, MCD, CPT-1, PFK, and PDK.

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198. Ahren, B. 2011. GLP-1 for type 2 diabetes. *Experimental cell research* 317:1239-1245.

Curriculum Vitae

Steven Paul Moberly

EDUCATION

Indiana University Southeast, New Albany, Indiana

2007, Bachelor of Science in Biology, and Associates of Arts in Chemistry

Indiana University, Indianapolis, Indiana

2012, Doctor of Philosophy, Cellular and Integrative Physiology

Indiana University School of Medicine, Indianapolis, Indiana

2014, Medical Degree, Medical Scientist Training Program

RESEARCH EXPERIENCE

2004 West Nile Virus Epidemiology and Public Health Laboratory, Student Researcher, Indiana University Southeast Department of Biology – Mentor: Claude Baker, Ph.D. (2004 to 2007)

2004 Botanical Molecular Biology Laboratory, Student Researcher, Indiana University Southeast Department of Biology – Mentor: Douglas Darnowski, Ph. D. (2004)

2006 Neuropsychopharmacology Laboratory, Undergraduate Research Fellow, Mayo Clinic Jacksonville – Mentor: Elliot Richelson, MD. (2006)

2007 Cellular Energetic Laboratory, Volunteer Student Researcher, University of Louisville School of Medicine, Department of Physiology and Biophysics – Mentor: William Ehringer, Ph.D. (2007-2008)

2008 Cardiovascular Pathophysiology Laboratory, Graduate Student Research, Indiana University School of Medicine, Department of Cellular and Integrative Physiology – Doctoral Thesis – Mentor: Johnathan Tune, Ph.D. – Co-Mentor – Kieren Mather MD. (2008-2012)

FUNDING AWARDS AND FELLOWSHIPS

2005 Eli Lilly Community Partners Program Fellowship with Clark County Health Department

2005 Indiana University SE Fall Fellowship for Research/Creative Work

2006 Mayo Clinic Jacksonville Summer Undergraduate Research Fellowship

2006 Indiana University SE Fall Fellowship for Research/Creative Work

2007 Indiana University SE Spring Fellowship for Research/Creative Work

2008 Indiana Medical Scientist Training Program Grant, Indiana University School of Medicine

PUBLICATIONS

Peer Reviewed Journal Articles

1. **Moberly SP**, Lalor C, McDonough M, Foster B, Estes A, Bentfield D. Discovery of an exotic asian mosquito species, *Ochlerotatus japonicus* (Diptera: Culiseta) in southern Indiana. *Indiana Academy of Science*. 2005; 114(1): 62-64

2. Darnowski DW, Celano M, **Moberly S**, Lalor C. Vegetative reproduction during development in Australian pygmy and tuberous sundews. *Acta Botanica Gallica.* 2005; 152(2): 147-157

3. Abarbanell AM, Merrmann JL, Weil BR, Wang Y, Tan J, **Moberly SP**, Fiege JW and Meldrum DR, Animal models of myocardial and vascular injury. *J Surg Res*. 2010; 162(2): 239-49

4. Berwick ZC, Dick GM, **Moberly SP**, Kohr MC, Sturek M, Tune JD. Contribution of voltage-dependent K(+) channels to metabolic control of coronary blood flow. *J Mol Cell Cardiol*. 2012; 52(4): 912-9

5. **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*. 2012; 237(3): 334-42

6. Berwick ZC, **Moberly SP**, Kohr MC, Morrical EB, Kurian MM, Dick GM, Tune JD. Contribution of voltage-dependent K(+) and Ca (2+) channels to coronary pressure-flow autoregulation. *Basic Res Cardiol*. 2012; 107(3): 1-11

Journal Articles in Preparation

1. **Moberly SP**, Mather KJ, Berwick ZC, Kohr MC, Hutchins GD, Green MA, Ng Y, Considine RV, Perry K, Chisholm RL, Tune JD. Impaired cardiometabolic responses to glucagon-like peptide in metabolic syndrome and type 2 diabetes mellitus

2. **Moberly SP**, Tune JD, Hutchins GD, Green MA, Considine RV, Perry K, Chisholm RL, Mather KJ. Effects of insulin on myocardial blood flow, total oxidative metabolism and fatty acid metabolism in lean and type 2 diabetic humans

3. Berwick ZC, Dick GM, **Moberly SP**, Kohr MC, Tune JD. Contribution of $Ca_V1.2$ channels to coronary microvascular dysfunction in metabolic syndrome

4. Kohr MC, Whitzmann FA, Lai X, Berwick ZC, **Moberly SP**, Obukhov AG, Tune JD. Effects of epicardial PVAT-derived factors on coronary vascular smooth muscle

5. Goodwill AG, Berwick ZC, Kohr MC, **Moberly SP**, Tune JD. Role of hydrogen sulfide in the coronary circulation

Published Abstracts Presented at National Meetings

1. **Moberly S** and Rajah T. Effects of Methoxychlor on Cell Survival and MAP Kinase Phosphorylation in Chinese Hamster Ovary Cells. *The American Society for Cell Biology 44th Annual Meeting*. 2004

2. **Moberly SP**. Student Directed West Nile Virus Program Generates New Mosquito Discoveries and Community Goodwill. *Council on Undergraduate Research Posters On The Hill*. 2006

3. **Moberly SP**, Berwick ZC, Kohr MC, Svendsen M, Mather KJ, Tune JD. Intracoronary Infusion of Glucagon-like peptide 1 acutely enhances myocardial glucose uptake during ischemia in canines. *Experimental Biology*. 2011

4. Berwick ZC, Kurian MM, Kohr MC, **Moberly SP**, Tune JD. Contribution of IK_{Ca} channels to the control of coronary blood flow. Experimental Biology. 2011

5. **Moberly SP**, Berwick ZC, Kohr MC, Mather KJ, Tune JD. Cardiac responses to intravenous glucagon-like peptide 1 are impaired in metabolic syndrome. *Experimental Biology*. In press

6. **Moberly SP**, Hutchins GD, Tune JD, Perry K, Chisholm RL, Considine RV, Mather KJ. Impaired myocardial response to glucagon-like peptide 1 in humans with Type 2 diabetes mellitus. *American Diabetes Association*. In press

7. NG Y, **Moberly SP**, Mather KJ, Hutchins GD, Green MA. Equivalence of arterial and venous blood for $^{11}CO_2$-metabolite analysis following intravenous administration of ^{11}C-acetate and ^{11}C-palmitate. *Society of Nuclear Medicine Annual Meeting*. In press

8. Berwick ZC, Dick GM, Bender SB, **Moberly SP**, Kohr MC, Goodwill AG, Tune JD. Contribution of $Ca_V1.2$ channels to coronary microvascular dysfunction in metabolic syndrome. *Experimental Biology*. In press

9. Berwick ZC, **Moberly SP**, Kohr MC, Morrical E, Kurian MM, Goodwill AG, Tune JD. Contribution of voltage-dependent potassium and calcium channels to coronary pressure-flow autoregulation. *Experimental Biology*. In press

10. Kohr MC, Lai X, **Moberly SP**, Berwick ZC, Witzmann FA, Tune JD. Augmented coronary vasoconstriction to epicardial perivascular adipose tissue in metabolic syndrome. *Experimental Biology*. In press

ACADEMIC HONORS AND AWARDS

2004 All-American Collegiate Scholar Award

2005 Lee Hamilton Scholarship

2005 Indiana University SE Full-time Academic Scholarship

2005 Indiana Environmental Health Association Scholarship

2005 Benjamin Cummings Scholarship

2005 Indiana University SE Outstanding Student Award

2006 Indiana University SE Outstanding Biology Student Award

2006 Council On Undergraduate Research Award

2006 President's Lifetime Achievement Volunteer Service Award

2006 Lee Hamilton Scholarship

2006 Harrison County Community Foundation Scholarship

2007 William B. Hebard Scholarship

2007 Chancellor's Medallion Scholarship Award

2008 Indiana University SE Outstanding Biology Student Award

TEACHING EXPERIENCE

2007 Anatomy Supplemental Instructor, Indiana University SE

2010 Case Study Instructor for Clinical Problem Solving, IUSM

2011 Case Study Instructor for Clinical Problem Solving, IUSM

PROFESSIONAL ORGANIZATIONS

Pre-Health Professional Society – Treasurer, Indiana University SE (2004-6)
Indiana University SE Biology Volunteers – Founder and President (2006-7)
Indiana Academy of Sciences – Student Member (2004-7)
American Physiological Society – Student Member (2010-12)

EXTRACURRICULAR ACTIVITIES

2007 Indiana University SE Biology Dept. Dr. Claude D. Baker Scholarship: A Call to Service from Steven P. Moberly – Founder

2010 IUSM Habitat for Humanity – Student Volunteer

2010 to 2011 IUSM Student Outreach Clinic – Student Volunteer