

Emerging Science Reviews

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Cardiovascular Actions of Incretin-Based Therapies

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Abstract: Glucagon-like peptide-1 receptor (GLP-1R) agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors represent 2 distinct classes of incretin-based therapies used for the treatment of type 2 diabetes mellitus. Activation of GLP-1R signaling or inhibition of DPP-4 activity produces a broad range of overlapping and unique cardiovascular actions. Native GLP-1 regulates cardiovascular biology via activation of the classical GLP-1R, or through GLP-1(9–36), a cardioactive metabolite generated by DPP-4-mediated cleavage. In contrast, clinically approved GLP-1R agonists are not cleaved to GLP-1(9–36) and produce the majority of their actions through the classical GLP-1R. The cardiovascular mechanisms engaged by DPP-4 inhibition are more complex, encompassing increased levels of intact GLP-1, reduced levels of GLP-1(9–36), and changes in levels of numerous cardioactive peptides. Herein we review recent experimental and clinical advances that reveal how GLP-1R agonists and DPP-4 inhibitors affect the normal and diabetic heart and coronary vasculature, often independent of changes in blood glucose. Improved understanding of the complex science of incretin-based therapies is required to optimize the selection of these therapeutic agents for the treatment of diabetic patients with cardiovascular disease. (*Circ Res.* 2014;114:1788-1803.)

Key Words: diabetes mellitus ■ dipeptidyl peptidase 4 ■ glucagon-like peptide 1 ■ heart ■ heart failure ■ myocardial ischemia

Incretin hormones are released from gut endocrine cells and potentiate meal-stimulated insulin secretion. Glucose-dependent insulinotropic polypeptide (GIP) was the first incretin hormone identified and is secreted from enteroendocrine K cells in the proximal jejunum. More than a decade later, the insulinotropic properties of GLP-1 were discovered.^{1,2} GLP-1(7–36) amide, the predominant secreted form, and GLP-1(7–37), collectively referred to as intact bioactive GLP-1, are synthesized in and secreted from enteroendocrine L cells distributed throughout the small and large intestines; however, the majority of intestinal GLP-1 content is localized to the distal gut.² GLP-1 is also produced in the central nervous system, primarily in the brain stem, and transported to multiple regions within the central nervous system. GLP-1 is constantly secreted from the intestine at low basal levels in the fasted and interprandial state, and circulating GLP-1 levels increase ≈2- to 3-fold after nutrient ingestion. Although the primary biological actions ascribed to intact GLP-1 are mediated by a single GLP-1 receptor

(GLP-1R),³ GLP-1 is rapidly cleaved by dipeptidyl peptidase-4 (DPP-4) into GLP-1(9–36; Figure 1), which exhibits its own biological activities (see below). Both intact GLP-1 and GLP-1(9–36) can be further cleaved by neutral endopeptidase 24.11, producing multiple smaller carboxyterminal peptides,⁴ including GLP-1(28–36), that may engage mitochondrial signaling pathways through poorly understood mechanisms (Figure 1). The biology of GLP-1 synthesis and secretion and the numerous metabolic actions of GLP-1 and GIP have been extensively reviewed elsewhere^{1,2} and will not be discussed further.

Native GLP-1, degradation-resistant GLP-1R agonists, and DPP-4 inhibitors exhibit numerous overlapping and distinct actions in the cardiovascular system^{5,6} (Table). Nevertheless, multiple new developments highlight the importance of re-evaluating current concepts of incretin action in the heart and vasculature. Published studies using nonspecific GLP-1R antisera for localization of GLP-1R expression in different cardiovascular tissues may not be correct.⁷ Unexpectedly,

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Nonstandard Abbreviations and Acronyms	
AMPK	5'AMP-activated protein kinase
ANP	atrial natriuretic peptide
CXCL12	chemokine (C-X-C motif) ligand 12
DPP-4	dipeptidyl peptidase-4
ERK	extracellular-regulated kinase
GIP	glucose-dependent insulinotropic polypeptide
GLP-1	glucagon-like peptide-1
HR	heart rate
LAD	left anterior descending
LVDP	left ventricular (LV) developed pressure
LVEF	LV ejection fraction
MI	myocardial infarction
SBP	systolic blood pressure
T2DM	type 2 diabetes mellitus
TAG	triacylglycerol
VSMC	vascular smooth muscle cell

the predominant localization of GLP-1R expression to atrial and not ventricular cardiomyocytes⁸⁻¹⁰ necessitates reconsideration of how GLP-1R agonists produce their actions on the heart. Finally, the differential cardiovascular actions of native GLP-1, its degradation products GLP-1(9-36) and GLP-1(28-36), and degradation-resistant GLP-1R agonists require clarification, in light of recent preclinical and clinical studies.

GLP-1R Agonists

Multiple GLP-1R agonists have been developed for the treatment of type 2 diabetes mellitus (T2DM; Figure 1). The first clinically approved agent, exenatide, is a synthetic version of the Gila monster salivary hormone, exendin-4, and is resistant to DPP-4-mediated degradation because of a position 2 glycine. Exenatide is delivered as a twice-daily injection or via a once-weekly microsphere-coupled formulation. Lixisenatide is a structurally related, DPP-4-resistant GLP-1R agonist for once-daily administration, encompassing exendin-4 devoid of its position 38 proline with 6 carboxyterminal lysine residues. Liraglutide is a long-acting, acylated, DPP-4-resistant human GLP-1 analog administered once daily, which noncovalently associates with albumin. Two high-molecular-weight GLP-1R agonists have been developed for once-weekly use. Albiglutide contains a GLP-1 dimer fused to albumin, with an alanine to glycine substitution at position 2 enabling DPP-4 resistance,¹¹ whereas dulaglutide is a DPP-4-resistant GLP-1R agonist with a modified immunoglobulin G fragment crystallizable region enabling extended pharmacokinetics. These agents are not metabolized to GLP-1(9-36) (Table; Figure 1), unlike native GLP-1, which may exert its actions through the GLP-1R or indirectly on cells that do not express the GLP-1R via its metabolites, GLP-1(9-36)/GLP-1(28-36) (Figure 2). Although liraglutide may be cleaved by DPP-4 and neutral endopeptidase 24.11, the major metabolites generated by metabolism of liraglutide are structurally distinct from those obtained after cleavage of native GLP-1.¹²

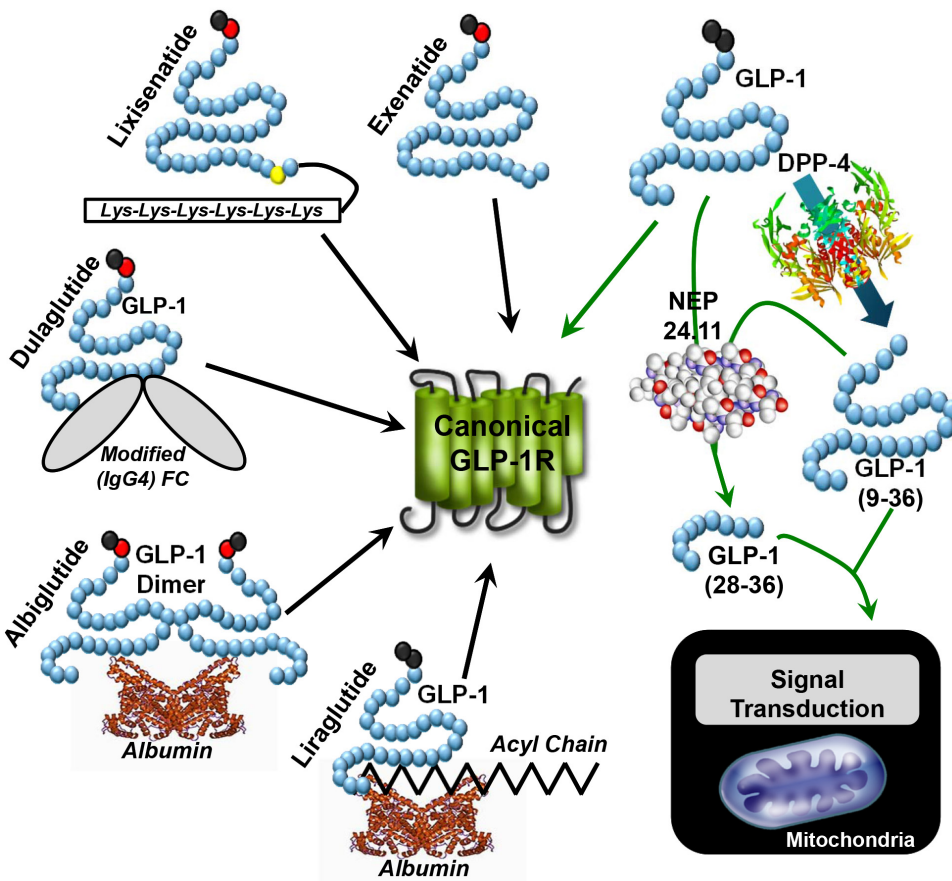


Figure 1. Glucagon-like peptide-1 (GLP-1) enzymatic cleavage, GLP-1 metabolites, and GLP-1 receptor (GLP-1R) agonists. Primary biological actions of GLP-1 pertain to the 30-amino acid GLP-1(7-36), which acts on the canonical G-protein-coupled GLP-1R to mediate its effects. However, GLP-1 is rapidly cleaved via dipeptidyl peptidase-4 (DPP-4) into GLP-1(9-36), which may exhibit its own distinct biological activity, potentially through direct effects on the mitochondria or incompletely identified signal transduction mechanisms. Both these forms of GLP-1 may also be cleaved via neutral endopeptidase (NEP) 24.11, generating multiple carboxyterminal fragments including the 9-amino acid GLP-1(28-36), which may undergo internalization into cells and target the mitochondria. In contrast, clinically used GLP-1R agonists are DPP-4-resistant and transduce their actions through the canonical GLP-1R.

Table. Contrasting Actions of Native GLP-1, GLP-1R Agonists, DPP-4 Inhibitors, and GLP-1(9–36) on the Cardiovascular System and Cardiovascular Risk Factors

	GLP-1R Agonists	GLP-1	DPP-4 Inhibitors	GLP-1(9–36)
LV function	Increased	Increased	Increased	Increased
Heart rate	Increased	Increased	No effect	No effect
Coronary flow	No effect	Increased	No effect	Increased
Infarct size	Decreased	Decreased	Decreased	Decreased
Body weight	Decreased	Decreased	No effect	No effect
Blood pressure	Decreased	Decreased	No effect/decreased	ND

The table depicts the effects of native GLP-1, GLP-1R agonists, GLP-1(9–36), and DPP-4 inhibitors on the parameters important for the cardiovascular system as inferred from available preclinical and limited clinical studies. Scant data from head-to-head clinical trials using these agents limit extrapolation of the available data to human subjects. DPP-4 indicates dipeptidyl peptidase-4; GLP-1R, glucagon-like peptide-1 (GLP-1) receptor; LV, left ventricular; and ND, not determined.

The GLP-1R

A single G-protein-coupled receptor with significant amino acid homology to related class B family G-protein-coupled receptors transduces the actions of GLP-1 linked to the control of glucose and body weight.^{2,13} Originally identified in islet

β -cells, the GLP-1R is widely expressed in extrapancreatic tissues, including lung, kidney, brain, the enteric and peripheral nervous system, lymphocytes, smooth muscle cells (SMCs), and atrial cardiomyocytes.^{8,14} However, the actions of GLP-1 and structurally related GLP-1R agonists have been reported

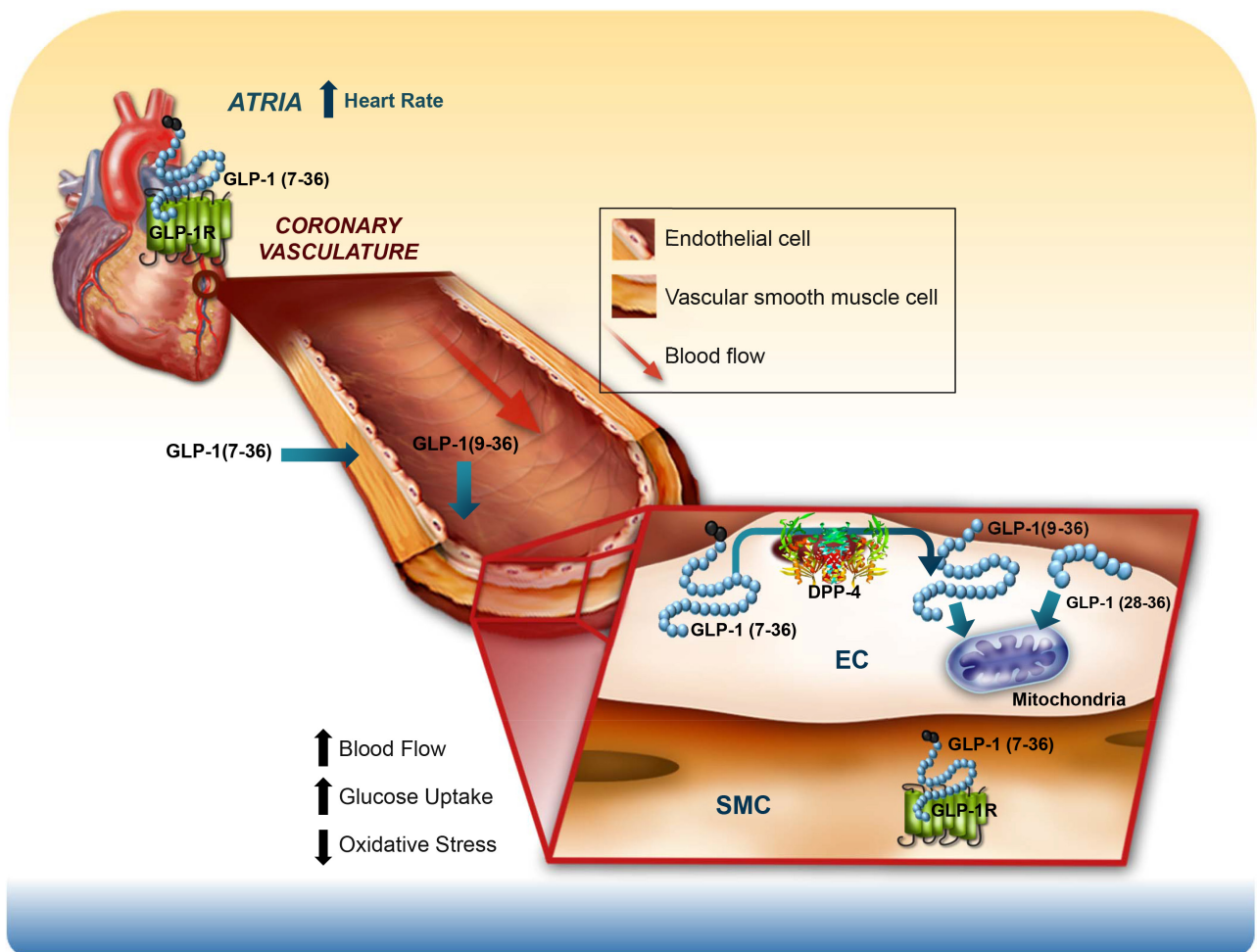


Figure 2. Actions of glucagon-like peptide-1 (GLP-1) and GLP-1 receptor (GLP-1R) agonists on the atria and vasculature. GLP-1R is localized to atrial cardiomyocytes and coronary vascular smooth muscle cells (VSMCs) in the heart. Furthermore, GLP-1-mediated increases in heart rate are consistent with functional atrial GLP-1R expression in pacemaker cells. The presence of GLP-1R on VSMCs, and dipeptidyl peptidase-4 (DPP-4) expressed in the endothelial cells (ECs) of coronary vasculature, suggests that a combination of GLP-1 and GLP-1(9–36) action on VSMCs and endothelial cells, respectively, may be responsible for GLP-1-mediated increases in coronary flow and subsequent myocardial glucose uptake.

in cells and tissues that do not express the canonical GLP-1R, through incompletely defined mechanisms.^{6,15}

Atrial GLP-1R Expression and Implications for GLP-1 Action on the Myocardium

Numerous studies demonstrate that GLP-1R agonists produce direct actions in the myocardium⁶; nevertheless, ventricular myocytes from multiple species do not express the *Glp1r*,^{8–10} raising important questions about mechanisms linking cardiac GLP-1R signaling to ventricular cardioprotection. Using complementary DNA generated from cultured atrial and ventricular cardiomyocytes of adult mice, full-length *Glp1r* mRNA transcripts were detected by reverse transcription polymerase chain reaction from mouse atrial but not ventricular myocytes.⁸ These findings have been corroborated independently in rats by the detection of *Glp1r* mRNA transcripts in RNA from isolated islets and the lung, but not in RNA isolated from ventricular cardiomyocytes and whole myocardial tissue extracts.¹⁶ Complementary studies analyzing transgenic expression of a yellow fluorescent reporter protein under the control of endogenous mouse *Glp1r* regulatory sequences failed to detect reporter expression in murine ventricular myocardium; however, cells positive for yellow fluorescence were scattered throughout the atrial myocardium.¹⁰ A novel mouse monoclonal antibody localized immunohistochemical GLP-1R expression to the atria, but not the ventricle, predominantly within the sinoatrial node, in monkey and human heart tissue.⁹ Taken together, these findings are consistent with an earlier report describing *Glp1r* expression in the right and left atria, but not the ventricle, of the mouse heart,¹⁷ although low levels of *Glp1r* expression in noncardiomyocyte cell types within the ventricles, in blood vessels, fibroblasts, immune cells, or nerves, cannot be excluded.

The recognition that *Glp1r* mRNA transcripts and GLP-1R protein are not detected in ventricular cardiomyocytes prompted the reassessment of earlier reports using polyclonal antisera to localize GLP-1R expression in the heart.¹⁸ More rigorous evaluation of commercially available GLP-1R antisera demonstrated a lack of sensitivity and problems with nonspecificity.⁷ Indeed, most GLP-1R antisera detect multiple nonspecific immunoreactive proteins in tissue extracts from *Glp1r*^{-/-} mice.⁷ Although *Glp1r* mRNA transcripts are detected in whole heart extracts,^{14,18,19} such methods do not distinguish between vascular SMCs (VSMCs) or atrial cardiomyocytes as the cellular source of *Glp1r* mRNA transcripts.^{9,10} Furthermore, technical difficulties in isolating pure cardiomyocyte populations from the ventricle independent of contaminating atrial cardiomyocytes from neonatal mouse hearts may explain alterations in signaling observed in experiments using GLP-1R agonists.²⁰ Because atria are predominantly kept intact during isolated ex vivo Langendorff heart perfusions, changes in left ventricular developed pressure (LVDP) after exposure to GLP-1R agonists may reflect contributions from atrial metabolites or proteins.⁸ Alternatively, GLP-1R agonists may directly influence cardiac performance via signaling through the VSMC GLP-1R,^{9,10} whereas the use of native GLP-1, or GLP-1(9–36), may trigger cardiomyocyte or vascular signaling pathways independent of the known

GLP-1R, which may be linked to increases in coronary flow or cardioprotection.^{18,21}

GLP-1–Mediated Cardiovascular Actions In Vitro/Ex Vivo

Glp1r is widely expressed in numerous organ systems^{2,14}; hence systemic GLP-1R activation may influence the cardiovascular system through both direct actions on the heart (Figure 2) and via indirect actions arising from GLP-1R signaling in peripheral tissues (Figure 3). Although clinically used GLP-1R agonists are not cleaved to produce GLP-1(9–36), the interpretation of data using native GLP-1 is complicated, because of the generation of GLP-1(9–36), which in turn may exert direct endothelial actions, facilitating increased blood flow (Table; Figure 2).^{20,22,23} Furthermore, DPP-4 inhibitors not only increase the levels of intact bioactive GLP-1 but also reduce the generation of GLP-1(9–36). The following sections will describe the direct and indirect actions of GLP-1R agonists on the heart in light of these important considerations.

Direct GLP-1 Action on the Cardiac Myocyte and Myocardium

Treatment of adult rat cardiomyocytes with native GLP-1 (10 nmol/L for 20 minutes) increased cyclic AMP levels, recapitulating the classical actions of GLP-1 in pancreatic β -cells.²⁴ Interestingly, the increase in cyclic AMP levels was not coupled to increased cardiomyocyte contractility as would be anticipated for a cyclic AMP–generating agent in the heart.²⁴ Treatment of neonatal mouse cardiomyocytes with exendin-4 (3 nmol/L for 20 minutes) increased Akt and extracellular-regulated kinase (ERK) phosphorylation.²⁰ Furthermore, exendin-4 reduced apoptosis after either 16 hours of hypoxia/4 hours reoxygenation (95% CO₂/5% O₂) or hydrogen peroxide stress (100 μ mol/L for 7 hours), actions prevented by the phosphatidylinositol-3 kinase inhibitor, LY294002, or the ERK inhibitor, PD98059.²⁰ Similarly, GLP-1 (200 nmol/L for 24 hours) activated phosphatidylinositol-3 kinase/Akt and ERK in HL-1 atrial cardiomyocytes, reducing the extent of apoptosis in response to staurosporine treatment; these cytoprotective effects were attenuated by the phosphatidylinositol-3 kinase inhibitor, wortmannin, or the ERK inhibitor, UO126.²⁵ Although these findings support the notion that native GLP-1 and degradation-resistant exendin-4 exert direct actions on cardiomyocytes, whether these actions are mediated by the classical GLP-1R is often not clearly determined. Furthermore, concentrations of native GLP-1 or exendin-4 used in these experiments are considerably higher (3 nmol/L to 200 nmol/L) than those observed physiologically after nutrient ingestion (20–30 pmol/L) or pharmacologically in therapeutic studies.

Perfusion of isolated Langendorff aerobic rat heart with GLP-1 (0.5 nmol/L) reduced LVDP,²¹ whereas GLP-1 (0.3 nmol/L) increased LVDP by \approx 20% during aerobic perfusion of isolated Langendorff mouse hearts.¹⁸ Despite these contradictory findings, GLP-1 consistently increased coronary flow and glucose uptake during aerobic perfusion in both rat and mouse hearts while also improving LVDP recovery during reperfusion after either low-flow or global no-flow ischemia.^{18,21} The GLP-1–induced increase in

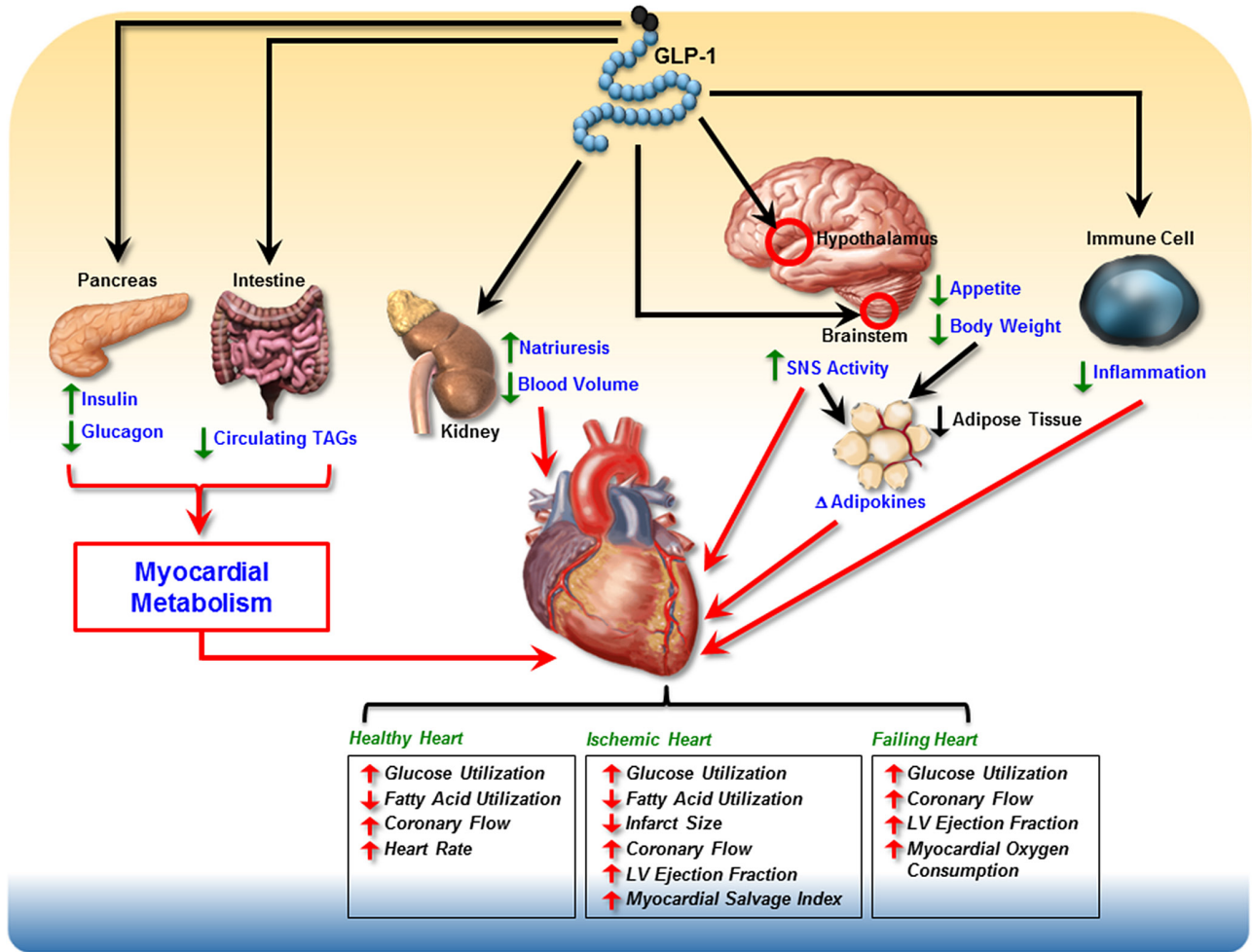


Figure 3. Potential indirect cardiovascular effects of glucagon-like peptide-1 receptor (GLP-1R) agonists. Direct actions of GLP-1 on islets result in elevated insulin and reduced glucagon levels, whereas GLP-1 action in the intestine has been associated with reductions in circulating lipids. These metabolic alterations decrease fatty acid delivery and enhance glucose uptake in the heart and may explain the increased glucose/decreased fatty acid utilization and enhanced left ventricular (LV) function observed in the myocardium after systemic GLP-1R activation. Direct actions on immune cells to decrease inflammation may also indirectly improve myocardial metabolism and subsequent contractile function. Furthermore, direct actions on the sympathetic nervous system (SNS) may contribute to GLP-1–induced alterations in heart rate, whereas the reduction in appetite after hypothalamic GLP-1R activation results in reductions in adiposity and body weight, which combined with the direct and indirect effects on the kidney to decrease blood volume (through increased natriuresis) likely contribute to the overall improvement in LV function after systemic GLP-1R activation. TAG indicates triacylglycerol.

myocardial glucose uptake seems independent of insulin signaling and is associated with increased p38 mitogen-activated protein kinase activity, enhanced nitric oxide (NO) production, and increased glucose transporter 1 protein levels at the sarcolemmal membrane.²¹ Conversely, both GLP-1 (0.5–5 nmol/L) and exendin-4 (0.5–5 nmol/L) failed to increase myocardial glucose uptake in isolated rat hearts perfused aerobically in the working mode in the presence of clinically relevant (0.4 mmol/L) concentrations of oleic acid.²⁶ Hence, the actions of native GLP-1 or GLP-1R agonists are not always reproduced across laboratories, which may reflect differences in experimental models and techniques. Regardless, the observations using native GLP-1 in ex vivo studies are entirely consistent with GLP-1(9–36)–mediated alterations in endothelial function and coronary flow and independent of direct actions on ventricular cardiomyocytes.

GLP-1 Signaling in Endothelial Cells

Direct treatment of human umbilical vein endothelial cells with liraglutide (0.1–100 µg/mL for 5 hours) increased endothelial NO synthase phosphorylation and NO production in a 5'AMP-activated protein kinase (AMPK)–dependent manner,²⁷ whereas both GLP-1 (100 nmol/L) and exendin-4 (10 nmol/L) increased endothelial NO synthase phosphorylation and NO production in human coronary artery endothelial cells.²⁸ Furthermore, treatment with GLP-1 (0.03 and 0.3 nmol/L for 4 hours) reduced reactive oxygen species and vascular cell adhesion molecule-1 mRNA expression in human umbilical vein endothelial cells after exposure to advanced glycation end products (100 µg/mL glycated bovine serum albumin).²⁹ Similarly, liraglutide (1 µg/mL) increased NO production, reduced tumor necrosis factor-α (10 ng/mL)–induced nuclear factor κB activation, and decreased the expression of inflammatory genes such as vascular cell adhesion

molecule-1 and monocyte chemoattractant protein-1 in human umbilical vein endothelial cells.²⁷ GLP-1(9–36) (0.3 nmol/L) improved human aortic endothelial cell viability in response to both simulated hypoxia (48 hours)/reoxygenation (7 hours) injury and hydrogen peroxide treatment (700 μ mol/L), actions prevented by treatment with L-NG-nitroarginine methyl ester (L-NAME; 150 μ mol/L), implicating a NO-dependent mechanism.²⁰ In contrast, exendin-4, which is not cleaved by DPP-4, does not improve human aortic endothelial cell viability after hydrogen peroxide treatment (700 μ mol/L), nor vasodilation in phenylephrine (3 μ mol/L)-precontracted mesenteric arteries.^{18,20} Because the majority of studies fail to demonstrate the expression of full-length GLP-1R in non-immortalized endothelial cells *in vivo*,^{9,10} it seems probable that clinically used degradation-resistant GLP-1R agonists such as exenatide, liraglutide, and lixisenatide do not directly influence endothelial function *in vivo*. In contrast, native GLP-1 acts primarily on endothelial cells via engagement of signal transduction pathways activated by GLP-1(9–36) or its metabolite GLP-1(28–36)³⁰ (Figures 1 and 2). Nevertheless, it remains possible that some immortalized endothelial cell lines or a subset of endothelial cells in specific vascular beds, such as the kidney,³¹ may express the canonical GLP-1R.

GLP-1R Signaling in VSMCs

Richards et al¹⁰ detected *Glp1r*-dependent reporter expression within ventricular blood vessels in cells that coexpressed smooth muscle actin, although the precise cardiac cell types expressing a fluorescent reporter protein under control of *Glp1r* regulatory sequences were incompletely characterized.¹⁰ Functionally, treatment of mouse aortic VSMCs with exendin-4 (10 nmol/L) reduced platelet-derived growth factor (25 ng/mL for 24 hours)-induced cell proliferation,³² and exendin-4 (5 nmol/L) rapidly reduced platelet-derived growth factor (10 ng/mL for 48 hours)-induced proliferation of rat aortic VSMCs in a protein kinase A-dependent manner.³³ Furthermore, liraglutide (0.01–1 μ g/mL for 24 hours) reduced oxidized low-density lipoprotein (10–40 μ g/mL for 24 hours)-induced mitochondrial reactive oxygen species generation in human aortic smooth muscle cells.³⁴ Overexpression of the lectin-like oxidized low-density lipoprotein scavenger receptor-1 abrogated liraglutide-mediated reduction in mitochondrial reactive oxygen species.³⁴ Therefore, the VSMC seems to be a direct target for GLP-1 action (Figure 2).

Reappraisal of Cardioprotective Actions of GLP-1

The atrial localization of GLP-1R expression necessitates reconsideration of how GLP-1R agonists exert their rapid cardioprotective actions.^{18,21,35} Although native GLP-1 and degradation-resistant GLP-1R agonists exert direct cardioprotective actions on cultured cardiomyocytes,^{20,36} it seems likely that GLP-1R-dependent protection of ventricular cardiomyocytes *in vivo* is indirect, achieved through mechanisms activated by either the atrial⁸ or vascular GLP-1R.¹⁰ Alternatively, changes in neural transmission or metabolism might also contribute to cardioprotection (Figure 3).

GLP-1 Action on the Vasculature and Lipid Metabolism

The following sections will describe the effects of GLP-1 and GLP-1R agonists on vascular and endothelial function in animals and humans while also describing the actions on other cardiovascular risk factors that may influence cardiac function, with a specific focus on dyslipidemia.

GLP-1 and Endothelial Function

Preclinical Studies

A vascular target for GLP-1 action in the heart is consistent with studies demonstrating GLP-1-mediated increases in microvascular and coronary blood flow.^{18,37,38} Sprague-Dawley rats infused with GLP-1 (30 pmol/kg per minute) exhibited significant increases in both microvascular blood volume and microvascular blood flow determined by contrast-enhanced ultrasound imaging.³⁷ These actions were dependent on NO production and prevented by infusion of the NOS inhibitor, L-NAME. Similarly, GLP-1 infusion increased coronary blood flow in dogs with pacing-induced dilated cardiomyopathy³⁹ and in the Langendorff aerobically perfused mouse heart.¹⁸ Consistent with the vasoactive properties of the GLP-1 metabolite GLP-1(9–36), native GLP-1, but not exendin-4, dilated blood vessels from *Glp1r*^{-/-} mice.¹⁸ Furthermore, infusion of GLP-1(9–36) (1.5 pmol/kg per minute) mimicked the effects of native GLP-1, with both peptides increasing coronary blood flow, myocardial glucose uptake, and LV systolic function in dogs with pacing-induced cardiomyopathy.²²

Clinical Studies

Analogous actions of native GLP-1 on vascular function have been observed in humans; GLP-1 infusion (1.2 pmol/kg per minute) in healthy volunteers (n=10) enhanced acetylcholine (2–8 μ g/100 mL)-induced forearm blood flow measured by venous occlusion plethysmography, whereas GLP-1 did not affect sodium nitroprusside-regulated blood flow (0.5–2 μ g/100 mL).⁴⁰ Moreover, fasted subjects with T2DM and stable coronary artery disease (n=12) showed improved endothelial function after GLP-1 infusion (2 pmol/kg per minute), as indicated by an increase in flow-mediated vasodilation of the brachial artery during a hyperinsulinemic clamp.⁴¹ In an observational study of 20 diabetic subjects on background metformin therapy, exenatide treatment (5–10 μ g twice daily) for 16 weeks improved flow-mediated vasodilation of the brachial artery after 5 minutes of forearm ischemia as determined via ultrasound echocardiography compared with patients treated with glimepiride (2–4 mg once daily).⁴²

It is unclear and doubtful whether the beneficial endothelial effects ascribed to native GLP-1 in humans are mediated through an endothelial GLP-1R. Many of these studies do not control for the effects of GLP-1 to increase insulin and reduce glucose, which in turn may indirectly improve endothelial function. Intra-arterial infusion of GLP-1 (20 pmol/kg per minute) in obese subjects with the metabolic syndrome improved acetylcholine- and sodium nitroprusside-enhanced forearm blood flow only in the presence of an intra-arterial infusion of insulin (0.1 mU/kg per minute).⁴³ In contrast, femoral artery infusion of GLP-1 (1 pmol/kg per minute) in overnight-fasted, healthy young men enhanced microvascular

recruitment assessed by contrast-enhanced ultrasound imaging, with or without coinfusion of 0.5 $\mu\text{g/mL}$ octreotide (to inhibit insulin secretion), indicating that the vascular effects of native GLP-1 in healthy subjects were independent of insulin.⁴⁴ Furthermore, GLP-1 promoted vasodilation of isolated precontracted (3 $\mu\text{mol/L}$ phenylephrine) mesenteric arteries *ex vivo* in the absence of insulin in a NOS-dependent manner.¹⁸ It seems likely that many findings attributed to native GLP-1 are mediated by GLP-1(9–36) or related degradation products that exert vasodilatory actions independent of the classical GLP-1R. Supporting this notion, degradation-resistant GLP-1R agonists, or DPP-4 inhibitors, do not consistently improve endothelial function. The effects of exenatide (3 μg twice daily for 1 month followed by 10 μg twice daily for the final 2 months) versus metformin (final dose 1000 mg twice daily) on microvascular endothelial function were assessed in obese, nondiabetic patients. No significant differences in digital reactive hyperemia were detected for either treatment group.⁴⁵ Surprisingly, treatment of diabetic Japanese subjects with the DPP-4 inhibitors sitagliptin (50 mg/kg per day) or alogliptin (25 mg/kg per day) for 6 weeks deteriorated endothelial function assessed by quantifying flow-mediated vasodilation of the brachial artery (7.2–4.3% postsitagliptin and 7.0–4.8% postalogliptin)⁴⁶ and is consistent with DPP-4 inhibition preventing the formation of GLP-1(9–36). Collectively, these findings necessitate a re-evaluation of the direct versus indirect actions of GLP-1R agonists versus native GLP-1 on endothelial and VSMCs in nondiabetic versus diabetic subjects (Figure 2; Table).

GLP-1 and Dyslipidemia

Preclinical Studies

Acute exendin-4 (24 nmol/kg intraperitoneally) treatment decreased postprandial triacylglycerol (TAG) and apolipoprotein B48 levels in C57BL/6J mice administered olive oil and Triton WR1339 after a 5-hour fast.⁴⁷ These effects were independent of gastric emptying and still observed if exendin-4 was administered 1 hour after the oral fat load.⁴⁷ Moreover, exendin-4 (0.1 nmol/L) decreased secretion of ³⁵S-labeled apolipoprotein B48 in hamster enterocyte cultures. Although chronic administration of GLP-1R agonists in rodent models of dyslipidemia also reduces the levels of circulating lipoproteins, whether these findings reflect direct or indirect actions on liver, intestine, or other tissues remains unclear.^{48,49} Furthermore, the specific GLP-1R⁺ cell types mediating these effects on intestinal and hepatic lipoprotein metabolism require further elucidation. Because intracerebroventricular exendin-4 administration (0.023 or 0.23 nmol) reduced hepatic TAG secretion after an overnight fast in C57BL/6 mice fed a high-fat diet for 4 weeks,⁷ and acute intracerebroventricular GLP-1 administration rapidly lowered hepatic TAG content in hyperinsulinemic mice fed a high-fat diet,⁵⁰ the importance of central GLP-1R signaling for the control of lipid metabolism requires further investigation.

Clinical Studies

Studies in subjects with T2DM demonstrated that the coadministration of GLP-1 (25 nmol subcutaneously directly before meals) with insulin for 5 days, followed by GLP-1

treatment for an additional 2 days, decreased plasma concentrations of very low-density lipoprotein TAG while increasing the size of low-density lipoprotein cholesterol particles.⁵¹ In healthy humans consuming a 250-kcal solid test meal, a GLP-1 infusion (1.2 pmol/kg per minute) for 6.5 hours inhibited the postprandial rise in plasma TAG and free fatty acid levels.⁵² In patients with T2DM, a 4-hour GLP-1 (1.2 pmol/kg per minute) infusion after a 10-hour fast resulted in a significant reduction in plasma-free fatty acid levels.⁵³ Similarly, a hyperglycemic clamp followed by a hyperinsulinemic–euglycemic clamp in elderly patients with T2DM treated for 3 months with a continuous GLP-1 infusion (100 pmol/kg per hour subcutaneously) also led to a reduction in plasma-free fatty acid levels during the hyperinsulinemic portion of the clamp, concomitant with a significant increase in plasma insulin levels.⁵⁴ Furthermore, a double-blinded crossover study in patients with recent-onset T2DM demonstrated that a single subcutaneous injection of exenatide (10 μg) reduced postprandial TAG and apolipoprotein B48 levels, as well as plasma remnant lipoprotein cholesterol, for \approx 8 hours in response to a high-calorie (600 kcal/m² of body surface area), fat-enriched (45%) breakfast meal after an overnight fast.⁵⁵ These effects were observed during the first 4 hours after the breakfast meal, before changes in plasma insulin levels. The design of many of these studies does not avoid the confounding effects of inhibition of gastric emptying on the delivery and absorption of ingested nutrients. However, studies in healthy men using nasoduodenal tubes to bypass the stomach and enable intestinal delivery of a high-fat, mixed macronutrient, liquid formula demonstrated that acute exenatide treatment (10 μg subcutaneously) 5 hours into the liquid meal infusion had no effect on hepatic production of apolipoprotein B100, but reduced TAG-rich apolipoprotein B48 lipoprotein production rates.⁵⁶ These findings, recapitulated in healthy nondiabetic human subjects acutely treated with sitagliptin,⁵⁷ suggest that the activation of GLP-1R signaling inhibits intestinal lipoprotein production in humans independent of gastric emptying.

GLP-1 and Hypertension

Although acute GLP-1R activation transiently increases blood pressure (BP) in animals,⁶ long-term treatment with GLP-1R agonists reduces BP in hypertensive subjects with T2DM or obesity, as discussed below.

Preclinical Studies of GLP-1–Mediated Alterations in BP

Chronic treatment with exendin-4 (20 nmol/kg twice daily) for 12 weeks reduced systolic BP (SBP) in *db/db* mice and attenuated the increase in SBP (\approx 10 versus \approx 25 mm Hg increase) in *db/db* mice administered 2% salt in their drinking water for 2 weeks.⁵⁸ Furthermore, C57BL/6J mice infused with angiotensin II (1 $\mu\text{g/kg}$ per minute) for 2 weeks and treated with twice-daily exendin-4 (20 nmol/kg) exhibited a significant reduction in SBP.⁵⁸ Although exendin-4 (10 nmol/L) prevented acute angiotensin II–induced ERK phosphorylation in kidney proximal tubular cells,⁵⁸ evidence for GLP-1R expression in kidney tubular cells is lacking.^{9,31} Conversely, GLP-1R signaling may modify angiotensin II–induced hypertension in rodents through direct actions on atrial cardiomyocytes

linked to the secretion of atrial natriuretic peptide (ANP).⁸ Treatment with liraglutide (30 µg/kg twice daily) in angiotensin II–induced hypertensive mice increased ANP secretion and reduced both SBP and diastolic BP.⁸ These effects were recapitulated in mice with transverse aortic constriction and abolished in angiotensin II–infused hypertensive *Glp1r*^{-/-} and *Nppa*^{-/-} mice.⁸ Nevertheless, because GLP-1R expression in primates seems restricted to the sinoatrial node,⁵⁹ the GLP-1R–ANP axis described in rodents may not be conserved in humans.

Clinical Studies in Hypertensive Patients Treated With GLP-1R Agonists

The majority of trials investigating the antidiabetic actions of GLP-1R agonists have reported reductions in BP. Twice-daily exenatide treatment for 12 weeks produced small but nonsignificant reductions in 24-hour SBP (mean 3.5 mm Hg decrease) and modest weight loss (1.8 kg) in diabetic subjects.⁶⁰ Results from the Diabetes Therapy Utilization: Researching Changes in A1C, Weight and Other Factors Through Intervention with Exenatide Once Weekly (DURATION-1) trial demonstrated that diabetic patients treated with exenatide continuously for 1 year had a significant reduction in SBP (3.8–6.2 mm Hg decrease).⁶¹ Approximately 50% of patients with a baseline SBP ≥130 mm Hg achieved a normal SBP at week 52. Consistent with these findings, 314 overweight patients administered exenatide (10 µg twice daily for 82 weeks) also experienced improvement in both SBP and diastolic BP.⁶² Similarly, in a study of 268 obese nondiabetic patients that completed a 20-week treatment with once-daily subcutaneous liraglutide (1.2, 1.8, 2.4, or 3.0 mg) followed by a nonblinded, 2-year extension (final dose of 3.0 mg), a reduction in SBP (mean 4.6 mm Hg decrease) was observed.⁶³ Taken together, the available data strongly support a reduction of BP in many hypertensive subjects treated with GLP-1R agonists.

Potential Mechanisms Linking GLP-1 to Reduction of BP

GLP-1R agonists frequently reduce body weight; however, the improvement in SBP with liraglutide appears rapidly, often before significant weight loss is observed.^{64,65} The pivotal localization of GLP-1R expression to atria and VSMCs indicates potential mechanistic sites of action. Although liraglutide administration for 12 weeks increased plasma ANP levels in obese subjects with T2DM,⁶⁶ a crossover study in 12 healthy, nonhypertensive young men demonstrated that a 2-hour infusion of native GLP-1 (1.2 pmol/kg per minute) increased urinary sodium excretion, but did not increase plasma pro-ANP levels.⁶⁷ Furthermore, the restricted expression of GLP-1R to the monkey and human sinoatrial node further diminishes the likelihood of a direct GLP-1R–ANP axis in humans.⁵⁹ Whether VSMC GLP-1R activation is linked to BP reduction in humans remains to be determined. Importantly, GLP-1R agonists do not reduce BP in nonhypertensive subjects, and hypotension has not been associated with GLP-1R agonists in clinical trials. How GLP-1R signaling mechanisms coupled to BP reduction are restrained or silenced in normotensive subjects is an important subject for future investigation.

GLP-1 and Ischemic Heart Disease

Extensive data from preclinical and clinical studies demonstrate that GLP-1R agonists exhibit cardioprotective actions.⁶ Nevertheless, many studies do not mechanistically distinguish between direct versus indirect actions of GLP-1R agonists, nor take into account the potential actions of GLP-1(9–36) on the cardiovascular system. In the next section, we highlight the potential mechanisms of action responsible for GLP-1R–dependent and GLP-1R–independent cardioprotection against ischemia/reperfusion injury and acute myocardial infarction (MI) in studies using GLP-1R agonists, native GLP-1, or GLP-1(9–36).

Preclinical Studies of Ischemia/Reperfusion Injury

Sprague–Dawley rats treated with either GLP-1 (4.8 pmol/kg per minute intravenously)³⁵ or albiglutide (subcutaneous injection for 3 days at 3 or 10 mg/kg per day)⁶⁸ exhibited a marked reduction in infarct size after temporary occlusion of the left anterior descending (LAD) coronary artery. The cardioprotective effects of albiglutide were associated with improvements in cardiac energetics, because in vivo ¹³C nuclear MR studies demonstrated a significant increase in myocardial carbohydrate oxidation and corresponding decrease in fatty acid oxidation, a metabolic profile associated with improved efficiency of contractile function.⁶⁸ These alterations in myocardial metabolism are consistent with GLP-1R–mediated increases in plasma insulin and reductions in glucagon, respectively.⁶⁹ Cardioprotective actions of GLP-1R agonists have also been demonstrated in large animal models; treatment with exenatide (10 µg intravenously and 10 µg subcutaneously 5 minutes before the onset of reperfusion and continued twice daily for 2 days) decreased infarct size, increased insulin levels, and improved LV systolic function in pigs subjected to 75 minutes of LAD coronary artery occlusion followed by 72 hours of reperfusion.⁷⁰ In contrast, liraglutide (10 µg/kg per day subcutaneously) administered to pigs for 3 days before temporary LAD coronary artery occlusion did not reduce infarct size or improve LV function.⁷¹

Although the results of several studies are consistent with indirect GLP-1R–dependent metabolic actions contributing to cardioprotection, considerable evidence also demonstrates direct actions of GLP-1R agonists in the isolated heart. Native GLP-1 (0.3–0.5 nmol/L) improved the reperfusion recovery of LVDP after either low-flow ischemia or a global no-flow ischemia, in isolated Langendorff perfused hearts.^{18,21} In contrast, treatment at the onset of reperfusion with liraglutide (30 nmol/L), which is relatively resistant to DPP-4–mediated cleavage,¹² did not improve the recovery of LVDP after global no-flow ischemia in the isolated Langendorff mouse heart⁷² or in pigs subjected to ischemia/reperfusion injury in vivo.⁷¹ Conversely, exendin-4 (5 nmol/L), which is highly resistant to DPP-4–mediated cleavage, enhanced LVDP recovery during global no-flow ischemia/reperfusion in the isolated mouse heart, although the effects were not as potent as those of native GLP-1.¹⁸ Unexpectedly, exendin-4 also modestly improved reperfusion recovery in ex vivo hearts from *Glp1r*^{-/-} mice. Furthermore, lixisenatide (0.3 nmol/L), a structurally related, DPP-4–resistant exendin-4 derivative, decreased infarct size in rats after LAD coronary artery occlusion ex vivo for 45

minutes followed by 2 hours of reperfusion, but did not increase coronary flow or LV contractility.¹⁶

Clinical Studies

The seminal observation that a 72-hour infusion of GLP-1 (1.5 pmol/kg per minute) initiated \approx 3.5 hours after coronary angioplasty within \approx 6.5 hours from symptom onset in patients undergoing acute MI improved LV ejection fraction (LVEF; $29\pm 2\%$ versus $39\pm 2\%$), and regional myocardial wall motion engendered significant interest in the cardioprotective actions of GLP-1.⁷³ Acute infusion of GLP-1 (1.2 pmol/kg per minute) 30 minutes before dobutamine stress echocardiography and continuing for 30 minutes into recovery in 14 patients with stable coronary artery disease and normal resting LV function prevented the development of postischemic myocardial dysfunction.⁷⁴ Moreover, the infusion of GLP-1 (1.2 pmol/kg per minute) after completion of the first balloon occlusion reduced LV dysfunction during dual-inflation balloon angioplasty in 20 nondiabetic patients with single-vessel coronary artery disease.⁷⁵

Lønborg et al⁷⁶ investigated the effects of a 6-hour exenatide or placebo infusion (mean plasma concentration of exenatide was 0.177 ± 0.069 nmol/L) initiated 15 minutes before reperfusion onset in 172 patients undergoing percutaneous coronary intervention to treat ST-segment elevation MI. Exenatide reduced infarct size relative to the ischemic area at risk and increased the myocardial salvage index assessed via cardiac magnetic resonance at \approx 90 days postinfusion. In contrast, exenatide-treated patients did not exhibit reduced mortality or improved LV contractility. Post-hoc analysis revealed that final infarct size was reduced in patients treated with exenatide (9 versus 13 g), provided that the infusion took place <132 minutes from first medical contact to balloon treatment.⁷⁷ Cardioprotection with exenatide was observed independent of glycemia in both nondiabetic and diabetic subjects.⁷⁸ Complementary evidence for cardioprotective actions of exenatide was obtained in a study of 58 patients with ST-segment elevation MI and thrombolysis in MI flow grade 0 randomized to receive either placebo or exenatide (10 μ g subcutaneous injection and 10 μ g intravenous bolus 5 minutes before percutaneous coronary intervention, followed by 10 μ g subcutaneous injection twice daily for the next 2 days). LVEF assessed via speckle tracking echocardiography at 6 months post-percutaneous coronary intervention was enhanced; serum levels of creatine kinase-MB and troponin I were decreased; and infarct size at 1 month post-percutaneous coronary intervention was reduced in exenatide-treated subjects.⁷⁹

GLP-1 and Ischemic Heart Disease in the Context of Atrial/VSMC GLP-1R Expression

Taken together, GLP-1 and GLP-1R agonists produce favorable effects on cardiac function in animal models of ischemia/reperfusion injury and in patients with coronary artery disease, findings originally attributed to direct effects on the ventricular myocardium. In light of recent evidence demonstrating negligible ventricular cardiomyocyte GLP-1R expression,^{8,10,59} these GLP-1R-mediated effects on ventricular myocardium are almost certainly indirect. Although results of human studies suggest possible cardioprotection,⁷⁶

whether such findings will translate to improvements in clinically meaningful end points (ie, time to first confirmed MI, mortality because of cardiovascular causes) in larger, randomized, placebo-controlled trials encompassing a wide range of subjects remains uncertain.

GLP-1 and Heart Failure

The majority of studies using GLP-1R agonists demonstrate salutary actions in heart failure, although these findings are also limited by the inability to distinguish between direct and indirect cardiac actions mediated through or independent of the canonical GLP-1R. The following section will highlight potential mechanisms of action of GLP-1R agonists in experimental and clinical heart failure.

Preclinical Studies in Models of Ventricular Dysfunction

Systemic GLP-1R activation often produces beneficial effects on the failing heart. In a canine model of 28-day, rapid right ventricular pacing–induced heart failure, a 48-hour infusion of GLP-1 (1.5 pmol/kg per minute) exerted insulin-like properties on the heart, increasing glucose uptake during a hyperinsulinemic–euglycemic clamp,³⁹ effects dependent on the activation of p38 mitogen-activated protein kinase and NO synthase.⁸⁰ In spontaneously hypertensive and heart failure–prone rats, a 3-month intraperitoneal infusion of GLP-1 (1.5 pmol/kg per minute) improved survival and prevented the decline in LV contractile function, findings associated with reduced cardiomyocyte apoptosis.⁸¹ These results are consistent with actions of GLP-1/GLP-1(9–36) on the vasculature, because GLP-1 infusion increased coronary flow and myocardial glucose uptake in both studies.^{39,81} Furthermore, these findings were recapitulated via 48-hour infusion of GLP-1(9–36; 1.5 pmol/kg per minute) in canines with heart failure after 28 days of rapid right ventricular pacing,²² highlighting the potential contribution of GLP-1(9–36) to actions emanating from the administration of native GLP-1.

The effects of liraglutide to improve ventricular function in mice⁷² may involve AMPK. A short course of liraglutide (30 μ g/kg intraperitoneally twice daily for 1 week) increased AMPK phosphorylation; reduced cardiac expression of *Nppa*, *Nppb*, and *Myh7*; and improved LV systolic function in mice with obesity-associated cardiomyopathy, findings abolished by administration of the AMPK antagonist, compound C (10 mg/kg intraperitoneally every other day for 1 week).⁸² Nevertheless, whether the beneficial cardiovascular actions of liraglutide reflect direct or indirect actions on the heart, vasculature, or peripheral tissues (Figure 3) remains unclear.

Clinical Studies

A 5-week infusion of GLP-1 (2.5 pmol/kg per minute) in 12 patients (8 with T2DM) with New York Heart Association class III/IV heart failure improved LVEF, oxygen consumption, and 6-minute walk distance times.⁸³ However, this was a single-center, nonrandomized trial whose results have yet to be reproduced in a larger cohort. Conversely, a 48-hour infusion of GLP-1 (0.7 pmol/kg per minute) in 15 humans with congestive heart failure but without diabetes mellitus produced no beneficial effects on LV function but increased heart rate (HR;

2 beats per minute) and diastolic BP (3 mmHg).⁸⁴ Albiglutide was assessed in studies \approx 3 months of duration in human subjects with New York Heart Association class II/III congestive heart failure; however, results have not yet been reported. More data are required before clear conclusions can be reached about potential benefits or risks of native GLP-1 or degradation-resistant GLP-1R agonists in human subjects with heart failure, and the results of ongoing cardiovascular outcome studies will likely shed further light on this important issue.⁶

GLP-1 and Heart Failure in the Context of Atrial/VSMC GLP-1R Expression

Current evidence for the beneficial effects of GLP-1 in the failing heart is entirely consistent with native GLP-1 exerting its actions largely through the generation of GLP-1(9–36), resulting in improvements to coronary flow. Whether intact native GLP-1 also enhances vascular and cardiac function in the failing heart through the VSMC GLP-1R is not clear. Similarly, the consequences of sustained atrial GLP-1R signaling in the failing heart require further scrutiny.

Cardiovascular Biology of DPP-4

DPP-4, originally described as a lymphocyte cell surface protein, CD26, is a transmembrane-spanning glycoprotein exopeptidase that cleaves dipeptides from the N-terminus of peptides or proteins immediately after a position 2 proline or alanine.⁸⁵ Within the heart, DPP-4 has been localized predominantly to endothelial cells.^{86,87} DPP-4 exists in 2 molecular forms that display proteolytic activity: a membrane-spanning protein with a short intracellular tail and a circulating protein bereft of the intracellular and membrane-spanning regions. The biology of DPP-4 is complex, with both the membrane-spanning protein and the soluble circulating form exerting biological activities independent of the catalytic activity of the enzyme.⁸⁵ The observation that DPP-4 cleaves GLP-1 at the N-terminus, followed by demonstrations that pharmacological inhibition or genetic inactivation of DPP-4 increases intact GLP-1 in the circulation,² definitively established DPP-4 as a critical regulator of GLP-1 bioactivity. Nevertheless, DPP-4 cleaves multiple cardioactive peptides⁶; hence, the ascertainment of mechanisms mediating the actions of DPP-4 inhibitors in the cardiovascular system is challenging.

Cardioprotective Actions of DPP-4 Inhibitors in Preclinical Studies

Acute administration of sitagliptin (20 mg/kg intraperitoneally) to nondiabetic mice improved the recovery of LVDP in hearts subsequently subjected to ex vivo ischemia/reperfusion injury.⁸⁸ Furthermore, isolated hearts from nondiabetic *Dpp4*^{-/-} mice also exhibited enhanced recovery of LVDP during ex vivo ischemia/reperfusion injury.⁸⁸ Pretreatment with sitagliptin for either 3 or 14 days in nondiabetic mice increased plasma GLP-1 levels and reduced infarct size after temporary LAD coronary artery occlusion in a protein kinase A–dependent manner.⁸⁹ Similarly, administration of the DPP-4 inhibitor, PFK275-055 (10 mg/kg per day), for 4 weeks to obese, nondiabetic, insulin-resistant Wistar rats decreased infarct size, but had no effect on cardiac output or coronary flow.⁹⁰

The majority of studies in young healthy rodents demonstrate that DPP-4 inhibition leads to cardioprotection, often in association with increased stem cell mobilization, thought to be secondary to enhanced levels of stromal cell–derived factor-1.⁹¹ Stromal cell–derived factor-1, also designated chemokine (CXC motif) ligand 12 (CXCL12), regulates stem cell homing, angiogenesis, and cardiomyocyte repair and survival^{92–94} through the CXC chemokine receptor type-4. CXCL12 also binds to a second receptor, CXC chemokine receptor type-7; however, the importance, if any, of CXC chemokine receptor type-7 for CXCL12 action in the injured heart remains uncertain.⁹⁵ The plasma levels of circulating CXCL12, derived from endothelial cells and cardiomyocytes at the site of ischemic injury, serve as a biomarker of cardiac injury in human subjects with MI.⁹⁶ Enhancing CXCL12 activity, by administration of CXCL12, by viral, cellular, or plasmid-mediated overexpression of CXCL12, or through inhibition of DPP-4 activity, resulted in cardioprotective actions in the setting of acute ischemic cardiac injury.^{93,97,98} Experimental and clinical diabetes mellitus may be associated with a reduced endothelial progenitor response to hypoxic injury⁹⁹; however, this defect is reversible on administration of CXCL12.^{99,100} Furthermore, plasma levels of CXCL12 and circulating levels of progenitor cells are increased in human diabetic subjects treated with DPP-4 inhibitor, sitagliptin.¹⁰¹ Given the extensive preclinical data linking CXCL12 activity to DPP-4 inhibitor–mediated cardioprotection,^{91,93,97,98,102,103} CXCL12 is widely viewed as a putative mediator of DPP-4 action in the heart.

GIP as a Potential Cardioactive DPP-4 Substrate

GIP is essential for the glucoregulatory actions of DPP-4 inhibitors¹⁰⁴; hence, understanding GIP biology in the cardiovascular system may have clinical relevance. Although the GIP receptor was detected in rat atrial and ventricular tissue,¹⁰⁵ no studies have reported the actions of GIP on the myocardium. Although studies in rodents have linked GIP receptor activation to increased adipogenesis, adipokine expression, and obesity,^{106–109} it seems doubtful that DPP-4 inhibition acts through GIP receptor signaling to produce similar actions in humans.² Endothelial cells and macrophages may also express the GIP receptor.^{105,110,111} The available data on the cardiovascular importance of GIP alone, or as a cardioactive substrate mediating the actions of DPP-4 inhibitors, are limited.

DPP-4 Inhibition and Myocardial Ischemia in Humans

Studies in nondiabetic humans with coronary artery disease and normal resting LV function demonstrated that acute treatment with sitagliptin (100 mg orally 2 hours before a 75-g oral glucose load) mitigated myocardial dysfunction during dobutamine stress echocardiography.¹¹² Moreover, administration of sitagliptin (100 mg orally for 4 weeks) attenuated myocardial stunning in response to dobutamine stress in patients with coronary artery disease, T2DM, and normal resting LV function.¹¹³ Plasma GLP-1 levels were increased and LV function improved, although baseline LV function was normal (LVEF, 55–60%) in these patients. In contrast to favorable results observed in short-term studies, data from longer clinical trials are less compelling. A clinical trial was initiated to assess

the putative link between CXCL12 activity, DPP-4 inhibition with sitagliptin, and preservation of ventricular function in nondiabetic and diabetic human subjects after revascularization for acute MI.¹¹⁴ Although the combination of sitagliptin and granulocyte colony-stimulating factor, initiated after MI, increased the number of circulating progenitor cells in human subjects, no significant differences in LVEF assessed by MRI were noted in subjects randomized to sitagliptin/granulocyte colony-stimulating factor after follow-up for 6 months.¹¹⁵

Similarly, results from the Saxagliptin Assessment of Vascular Outcomes Recorded in Patients with Diabetes Mellitus-Thrombolysis in Myocardial Infarction 53 (SAVOR-TIMI 53) and Examination of Cardiovascular Outcomes with Alogliptin versus Standard of Care (EXAMINE) cardiovascular outcome trials demonstrated that DPP-4 inhibitors saxagliptin and alogliptin did not reduce cardiovascular events. SAVOR-TIMI 53 enrolled diabetic subjects with significant cardiovascular risk (history of multiple risk factors or previous acute coronary event before randomization). Treatment with saxagliptin or placebo was initiated with a median follow-up of 2.1 years. The EXAMINE study enrolled diabetic subjects with a recent acute MI or unstable angina requiring hospitalization within the previous 15 to 90 days. Study subjects were randomized to alogliptin or placebo, with a median duration of treatment of 533 days. No differences in the rates of MI or death from cardiovascular causes were observed in subjects treated with saxagliptin¹¹⁶ or alogliptin.¹¹⁷ Unexpectedly, a small but significant increase in the rates of hospitalization for heart failure was observed in patients randomized to saxagliptin.¹¹⁶ Hence, current evidence does not support the notion that DPP-4 inhibitors are cardioprotective in human subjects with pre-existing cardiovascular disease.

DPP-4 Inhibition and Heart Failure

Findings of increased hospitalization rates for heart failure in a small subset of saxagliptin-treated subjects contrast with results obtained in animal models of heart failure, wherein DPP-4 inhibition either had no effect or improved LV function. Administration of sitagliptin (30 mg/kg once daily) for 3 weeks to nondiabetic pigs with pacing-induced heart failure decreased HR, increased stroke volume, and preserved glomerular filtration rate,¹¹⁸ whereas diabetic mice treated with sitagliptin (250 mg/kg per day in the diet) for 12 weeks exhibited improved survival after permanent LAD ligation.⁸⁸ Furthermore, induction of heart failure in Wistar rats using radiofrequency catheter ablation increased plasma and myocardial membrane DPP-4 protein expression and activity that negatively correlated with LVEF and diastolic function.¹¹⁹ Intriguingly, treatment with sitagliptin (40 mg/kg twice daily via oral gavage) for 6 weeks abrogated impairment in both LV systolic and diastolic function associated with a \approx 3-fold increase in plasma GLP-1 levels; reduced myocardial fibrosis, cardiomyocyte hypertrophy, and apoptosis; and improved renal function.¹¹⁹ Using abdominal aortic banding to induce heart failure, *Dpp4*^{-/-} rats exhibited less attenuation of LV systolic function, ventricular fibrosis, and cardiomyocyte hypertrophy, assessed at both 6 and 10 weeks post-abdominal aortic banding compared with Fischer 344 *Dpp4*^{+/+} rats, findings associated with increased plasma GLP-1 levels.¹²⁰ Treatment of 6-week-old *db/db* mice with sitagliptin

(16 mg/day orally) for 4 weeks had no effect on LV function, but reduced AMPK/acetyl-CoA carboxylase ACC phosphorylation and myocardial fibrosis and decreased CD36 protein expression at the sarcolemmal membrane, suggesting that DPP-4 inhibition may reduce myocardial fatty acid uptake and subsequent fatty acid metabolism.¹²¹ Vildagliptin (10 mg/kg per day) administered to nondiabetic C57BL/6J mice for 4 weeks starting 1 day after transverse aortic constriction increased plasma GLP-1 levels, improved LV contractile function, reduced cardiomyocyte apoptosis and ventricular fibrosis, and improved survival.¹²² In contrast, vildagliptin (15 mg/kg per day) administered to nondiabetic rats either before or after permanent LAD ligation did not affect LV function or infarct scar formation despite increasing plasma GLP-1 levels.¹²³ Hence, preclinical data do not explain how chronic DPP-4 inhibition may increase the risk for heart failure in a subset of diabetic subjects. Reasons for the discrepancies between preclinical studies and the results from the SAVOR-TIMI 53 trial may include the small number, age, and good health of animals studied; importance of coadministered medications in the human heart failure population; species-specific differences; or other factors not yet identified.

The effects of vildagliptin on ventricular function have been studied in 254 diabetic subjects with New York Heart Association class I to III, mean age 63 years, and average body mass index 29 kg/m², with the proportion of New York Heart Association class I, II, and III being 9.8%, 52.8%, and 37.4%, respectively. Exposure to vildagliptin was not associated with overt impairment in LVEF; however, increases in LV end-diastolic and end-systolic volumes were reported and consistent with the possibility that DPP-4 inhibition leads to subtle deterioration of ventricular function in some subjects.¹²⁴ Whether a subset of subjects treated with both DPP-4 inhibitors and angiotensin-converting enzyme inhibitors exhibits increased vascular generation of catecholamines and deterioration of ventricular function secondary to potentiation of substance P action¹²⁵ requires further investigation.

Limitations and Future Directions

The cardioprotective actions of GLP-1R agonists and DPP-4 inhibitors in preclinical models of ischemic injury and heart failure are contrasted with modest and often inconclusive results with these agents in short-term human studies. The majority of preclinical studies with native GLP-1 or GLP-1R agonists demonstrate results compatible with cardioprotection; however, underlying mechanisms are frequently not well defined.⁶ Although the GLP-1R antagonist exendin(9–39) has been used to infer GLP-1R-dependent cardioprotection,¹²⁶ this peptide is not a highly selective GLP-1R antagonist.^{127,128} Indeed, exendin(9–39) is an inverse agonist¹²⁹ and blocks the cardioprotective actions of GLP-1(9–36) through pathways independent of the known GLP-1R.²⁰ Hence, definitive ascertainment of GLP-1R-dependent mechanisms using only exendin(9–39) may be challenging.

GLP-1R agonists increase HR in animals¹³⁰ and humans.⁶ The mechanisms for the increase in HR are poorly understood and may involve direct activation of sinoatrial GLP-1R, central or local augmentation of sympathetic nervous system activity, inhibition of parasympathetic nervous system inputs, or other

contributing factors. Whether increases in HR are sustained in diabetic subjects chronically treated with GLP-1R agonists remains unclear. Furthermore, whether short-acting versus long-acting GLP-1R agonists differentially affect HR in a clinically meaningful manner is not known. In contrast, DPP-4 inhibitors are not associated with increases in HR, probably because of lower levels of GLP-1 achieved with DPP-4 inhibition. Although BP reduction is frequently observed with chronic administration of GLP-1R agonists,^{6,131} DPP-4 inhibitors are associated with more modest reduction or no changes in BP. The multiple differences in cardiovascular mechanisms of action (Table) between GLP-1R agonists and DPP-4 inhibitors⁶ emphasize the importance of individual clinical trials to examine the cardiovascular impact of each agent.

Results from preclinical and clinical studies have raised important mechanistic questions. Is GLP-1R expressed in some, most, or an anatomically distinct subset of coronary arteries? Is vascular GLP-1R predominantly limited to SMCs, or do some endothelial cells express a functional GLP-1R? Does the expression and function of atrial or vascular GLP-1R change with age, or the development of diabetes mellitus, atherosclerosis, hypertension, ischemia, or impairment of ventricular function?

Although 2 outcome studies have not detected evidence for cardioprotection with DPP-4 inhibitors, both SAVOR-TIMI 53 and EXAMINE enrolled subjects with advanced cardiovascular disease who were exposed to a DPP-4 inhibitor for only 18 to 24 months.^{116,117} In contrast, other studies such as the Trial Evaluating Cardiovascular Outcomes with Sitagliptin (TECOS) will examine the cardiovascular safety of DPP-4 inhibitors in a population treated for much longer periods of time.¹³² Similarly, the Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results (LEADER) trial¹³³ will study a broader cross-section of diabetic subjects, some at an early stage of disease progression, for a longer period (≈ 5 years) of time. Furthermore, outcome studies with GLP-1R agonists will study different molecules with distinct structures and pharmacokinetic properties, as well as exhibit fundamental differences in trial design. Given the substantial differences in mechanism(s) of action between GLP-1R agonists and DPP-4 inhibitors (Table), these 2 classes of medications and their individual outcome studies should be viewed as completely distinct, and not highly inter-related.

The finding that native GLP-1 and GLP-1(9–36) enhance coronary flow is intriguing yet of uncertain clinical significance. Are the physiological levels of GLP-1(9–36) sufficient to control blood flow under basal conditions? Does the inhibition of DPP-4 activity, and thereby reduced generation of GLP-1(9–36), have meaningful consequences for cardiovascular function? How do GLP-1(9–36) and GLP-1(28–36) exert their pleiotropic actions and regulate mitochondrial signal transduction pathways? Is GLP-1 the dominant cardioactive DPP-4-sensitive substrate, or should we continue to investigate the putative importance of substance P, brain natriuretic peptide, neuropeptide Y, CXCL12, bradykinin, or related peptides regulated by DPP-4 activity?⁷⁶ Moreover, which animal models are best suited for mechanistic studies of incretin biology that may be translationally relevant to diabetic subjects with pre-existing cardiovascular disease? The results from ongoing

cardiovascular outcome trials for GLP-1R agonists and DPP-4 inhibitors⁶ will provide valuable new insights about the use and safety of different incretin-based therapies and will almost certainly raise new questions surrounding the mechanisms of incretin action in the diabetic cardiovascular system.

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