

Review Article

Cardiovascular consequences of drugs used for the treatment of diabetes: potential promise of incretin—based therapies

Kiwon Ban, MSc^{a,b,f}, Sonya Hui, BSc^{a,b,f}, Daniel J. Drucker, MD^{c,d,e},
and Mansoor Husain, MD^{a,b,c,f,*}

^aHeart & Stroke Richard Lewar Centre of Excellence for Cardiovascular Research, University of Toronto;

^bDepartment of Physiology, University of Toronto;

^cDepartment of Medicine, University of Toronto;

^dBanting & Best Diabetes Centre, University of Toronto;

^eSamuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto; and

^fToronto General Hospital Research Institute, Toronto, Ontario, Canada

Manuscript received December 5, 2008 and accepted April 6, 2009

Abstract

Cardiovascular disease is the predominant cause of death in diabetic patients, and yet the cardiovascular benefits of traditional drug treatments for hyperglycemia have been elusive. Two new classes of diabetic drugs targeting the glucagon-like peptide-1 (GLP-1) incretin pathway have emerged. The GLP-1 receptor agonists reduce blood glucose levels by stimulating insulin and inhibiting glucagon secretion and gastric emptying. Dipeptidyl peptidase-4 (DPP-4) inhibitors prolong the half-life of endogenous GLP-1 by inhibiting its proteolytic degradation to the metabolite GLP-1(9-36), thereby increasing insulin and reducing glucagon secretion. Here, we review the biology of GLP-1, including studies of GLP-1 in animal models and humans with heart disease. We also highlight the emerging salutary cardiovascular effects of both GLP-1 and GLP-1(9-36). Unlike the GLP-1R agonist *Exendin-4*, both GLP-1 and GLP-1(9-36) exert vasodilatory actions on coronary and peripheral mouse vessels. Importantly, the effects of GLP-1 on isolated hearts undergoing experimental ischemia and precontracted mesenteric arteries were reduced but not abolished by the DPP-4 inhibitor Sitagliptin. We posit that GLP-1-based therapeutics represent novel and promising anti-diabetes drugs, the direct cardiovascular actions of which may translate into demonstrable clinical benefits on cardiovascular outcomes. *J Am Soc Hypertens* 2009;3(4):245–259. © 2009 American Society of Hypertension. All rights reserved.

Keywords: Glucagon-like peptide-1 (GLP-1); ischemia-reperfusion injury; diabetes mellitus; insulin resistance.

Introduction

The Pandemic of Diabetes

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, action, or both.¹ Processes involved in

the development of diabetes range from autoimmune destruction of β cells (type 1) to abnormalities of β -cell function and insulin resistance (type 2).¹ The number of patients suffering from diabetes worldwide was ~170 million in 2000, and is predicted to exceed 300 million by 2025.² Although India and China exhibit the highest global prevalence of diabetes,² it is estimated that more

This study was supported in part by an Operating Grant from the Heart & Stroke Foundation of Ontario (HSFO; NA5926) awarded to Drs. Husain and Drucker; the Canada Research Chairs Program (Dr. Drucker) and a Career Investigator Award from the HSFO (Dr. Husain) (CI-5503).

Dr. Drucker has served as an advisor or consultant within the past 12 months to Amgen Inc, Amylin Pharmaceuticals, Arisaph Pharmaceuticals Inc, Chugai Inc, Conjuchem Inc, Eli Lilly Inc, Emisphere Technologies Inc, GlaxoSmithKline, Glenmark Pharmaceuticals, Isis Pharmaceuticals Inc, Johnson & Johnson, Merck

Research Laboratories, Novartis Pharmaceuticals, Novo Nordisk Inc, NPS Pharmaceuticals Inc, Phenomix Inc, Takeda, and Transition Pharmaceuticals Inc. Dr. Husain has served as a consultant within the past 12 months to Merck Research Laboratories.

Conflict of interest: none.

*Corresponding author: Mansoor Husain, MD, Toronto General Hospital Research Institute 200 Elizabeth Street, TMDT3-909, Toronto, Ontario, Canada M5G-1C4. Tel: 416-581-7488; fax: 416-340-4021.

E-mail: mansoor.husain@utoronto.ca

than 9% of the general U.S. population has diabetes,³ with another 26% exhibiting impaired glucose tolerance. As such, a staggering 35% of Americans manifest impaired glucose homeostasis. Given the adverse consequences of hyperglycemia on health, these prevalence rates attest to a significant and worsening public health issue, burdening health care resources in both the developed and developing world.

Diabetes and Cardiovascular Disease

Patients with diabetes are at increased risk for cardiovascular disease (CVD). Indeed, CVD is the predominant cause of death in diabetic patients,⁴ with complications such as myocardial infarction and stroke causing 75% of all deaths.² Compared with nondiabetic controls, diabetic patients have a several-fold increased risk of developing CVD.⁵ Type 1 patients possess a 10 times higher risk than the general population,⁶ whereas type 2 patients have a 3 times higher risk of cardiovascular death.⁷ In general, diabetic patients have a 3 to 5 times increased incidence of death from coronary heart disease compared with nondiabetic subjects, a risk level that equates with nondiabetic patients who have already suffered a myocardial infarction.^{8,9}

Why does this occur? Evidence suggests that the high prevalence of heart disease among diabetic patients is due to the deleterious effects of hyperglycemia on cardiovascular pathophysiology. Studies show that the association between CVD and blood glucose level is linear, there being no threshold level that emerges as “high-risk.”^{10–12} Blood glucose levels below the diabetic and impaired glucose tolerance ranges continue to have negative effects on cardiovascular outcomes.^{10–12} This may be due to the role played by chronic hyperglycemia on the development of atherosclerosis through cellular and molecular abnormalities in multiple relevant cell types.⁷ Pathogenic mechanisms linking high blood glucose levels with atherosclerosis include endothelial cell dysfunction, increased generation of glycosylated products, oxidative stress, and impaired vasodilation. Collectively, these abnormalities increase the occurrence and severity of thrombosis, vessel narrowing, and occlusion.^{7,12} Therefore, in addition to the microvascular dysfunction caused by diabetes (retinopathy, nephropathy, and neuropathy),² hyperglycemia accelerates macrovascular pathologies such as atherosclerosis and contributes to sequelae such as myocardial infarction, stroke, peripheral vascular disease, and heart failure.^{6,7,13}

Diabetes often coexists with other well-established risk factors for CVD such as hypertension and dyslipidemia. Individually, hypertension is the most common cardiovascular risk factor¹⁴ and a modifiable risk factor with great impact on CVD burden and mortality.¹⁵ Fortunately, several studies have demonstrated that pharmacological control of blood pressure (BP) leads to reductions in harmful cardiovascular

outcomes such as stroke and myocardial infarction, especially in circumstances of secondary prevention.^{16,17} Similarly, dyslipidemia is another key risk factor for CVD in which the management of blood lipid levels is effective in improving cardiovascular outcomes,^{18,19} particularly in survivors of an acute coronary event.²⁰ However, in stark contrast to the indisputable cardiovascular benefits of treating hypertension and dyslipidemia, the benefits of lowering blood glucose to reduce macrovascular disease have been difficult to demonstrate.²¹

Cardiovascular Effects of Drugs Commonly Used for the Treatment of Diabetes

Although diabetes commonly coexists with hypertension and dyslipidemia, forming the multifactorial disease complex of “metabolic syndrome” (including visceral obesity, hyperinsulinemia, endothelial dysfunction, and impaired fibrinolysis),⁷ the negative cardiovascular effects of hyperglycemia exist independently of other associated metabolic risk factors.¹² Indeed, considering the effects of elevated blood glucose levels on the cardiovascular system, interventions that aim to limit hyperglycemia *should* have a positive effect on cardiovascular outcomes. And yet, reduction of blood glucose has been unable to produce meaningful improvements in cardiovascular outcomes such as myocardial infarction or stroke. For instance, the United Kingdom Prospective Diabetes Study (UKPDS) did not find an association between glycemic control and a decrease in adverse macrovascular events.²²

Surprisingly little is known about the long-term effects of anti-diabetic agents on the cardiovascular system. Indeed, recent studies have shown that several groups of currently available diabetic drugs cause specific concerns, which may explain, in part, the lack of improved cardiovascular outcomes from the treatment of diabetes using specific agents.²¹

A table summarizing our review of mechanistic literature that may underlie this conundrum is provided (Table).^{23–36} Herein, we briefly expand on cardiovascular outcomes in diabetic patients taking 3 “traditional” commonly prescribed classes of oral anti-diabetic drugs: sulfonylureas, biguanides, and thiazolidinediones.

Sulfonylureas and Potassium Channels

Sulfonylureas have been prescribed for the treatment of type 2 diabetes for more than 50 years and include agents such as glibenclamide and glimepiride.³⁷ Sulfonylureas act on pancreatic islet β cells to stimulate insulin release by binding to the β -cell sulfonylurea receptor (SUR-1) and blocking adenosine triphosphate (ATP)-dependent potassium channels. This in turn leads to a calcium ion influx and activation of calcium-dependent proteins that regulate insulin secretion (see also glucagon-like peptide-1

Table

Cardiovascular effects of anti-diabetic drugs

Drug	Protective effect		Mechanism of action	References
	Nondiabetic	Diabetic		
Insulin	+	+	Survival kinases; opens mitochondrial K_{ATP} channel; pharmacological preconditioning	Libby et al ³¹ Sack et al ³⁵
Sulfonylureas	–	–	Closes mitochondrial K_{ATP} channels; inhibits ischemic preconditioning	Brady et al ²³ Garratt et al ²⁷
Biguanides (metformin)	±	±	Activates AMPK; increases glucose uptake; induces platelet thrombosis and lactic acidosis	Davis et al ²⁶ Olsson et al ³³ Calvert et al ²⁴
α -Glycosidase inhibitors	?	–	Impairs glucose homeostasis in ischemic hearts	Liao et al ³⁰
Rosiglitazone Pioglitazonew	+	±	PPAR γ agonists have anti-inflammatory effects; increase glucose uptake; increase fluid retention;	Lautamaki et al ²⁹ Home et al ²⁸ Couzin ²⁵
Glinides	±	±	Antioxidants; close mitochondrial- K_{ATP} channel; proarrhythmic	Quast et al ³⁴
GLP-1 analogues	+	+	Promotes ischemic preconditioning; increases glucose uptake; NO-dependent vasorelaxation	Nikolaidis et al ³² Sokos et al ³⁶ Ban et al ¹⁰⁰
DPP-4 inhibitors	?	?	Unknown	
Amylin analogues	?	?	Unknown	

DPP-4, dipeptidyl peptidase-4; GLP-1, glucagon-like peptide-1; AMPK, adenosine monophosphate-activated protein kinase; NO, nitric oxide; PPAR γ , peroxisome proliferator-activated receptor.

[GLP-1]'s mechanism of action in the following section).^{38,39} A recent large-scale analysis of the Diabetes Audit and Research in Tayside Scotland (DARTS) diabetes information system and the Medicines Monitoring Unit (MEMO) revealed that patients receiving sulfonylurea treatment, either alone or in combination with metformin, exhibited significantly increased cardiovascular morbidity and mortality as well as all-cause mortality compared to patients treated with metformin alone.⁴⁰ Another study assessing diabetic patients after angioplasty for acute myocardial infarction showed a 13% increase of in-hospital mortality in the sulfonylurea treatment group as compared with patients not on sulfonylureas.²⁷ However, the 2003 LAngendreer Myocardial infarction and Blood glucose in Diabetic patients Assessment (LAMBDA) Study of type 2 diabetics demonstrated that, compared with other anti-diabetic agents, sulfonylurea treatment before acute myocardial infarction did not cause a significant difference in survival.⁴¹

Potentially negative cardiovascular outcomes related to sulfonylurea use may be accounted for by studies suggesting that some sulfonylureas can impair ischemic preconditioning in the cardiac myocardium.²¹ Both in vivo and in vitro studies have implicated the importance of potassium-ATP channels in cardioprotection from ischemic injury.⁴² Sulfonylurea receptor isoforms that bind sulfonylurea have recently been discovered in the heart and vasculature,³⁰ and multiple studies using animal models suggest

that such ligand-receptor interactions may impair ischemic preconditioning responses.^{43–45} Indeed, the use of sulfonylureas has been correlated with impaired contractile function after ischemia in both animals and humans,^{46,47} as well as increased infarct size compared to saline-treated controls.⁴⁸

Of interest, recently published findings from the Action in Diabetes and Vascular Disease: Preterax and Diamicon Modified Release Controlled Evaluation (ADVANCE) and UKPDS have shown that sulfonylurea treatment had no significant effects on macrovascular events after an average 5- or 10-year follow-up, respectively.^{49,50} Therefore, additional research clarifying the link between long-term sulfonylurea use and cardiovascular outcomes is needed, because the current clinical evidence is limited and inconsistent.

Biguanides Are not Bygones

The biguanide class of oral hypoglycemic agents includes metformin, one of the most widely prescribed medications for type 2 diabetes.^{37,51} These are insulin-sensitizing compounds that act by reducing gluconeogenesis in the liver, enhancing insulin receptor expression on myocytes and adipocytes, and increasing glucose uptake.³⁷ Some studies have reported a cardioprotective effect of metformin in diabetic patients, but these findings remain controversial.⁵² Although the UKPDS reported modestly improved cardiovascular outcomes in obese patients

receiving metformin monotherapy, it also noted findings of increased cardiovascular-related mortality in patients taking a combination of metformin and a sulfonylurea.⁵³ Another study demonstrated increased mortality among type 2 diabetic patients taking metformin and a sulfonylurea in combination as compared with those on sulfonylurea treatment alone.³³ Moreover, a 1999 study that followed diabetic patients with coronary heart disease over a 5-year period found that metformin monotherapy or combination metformin/sulfonylurea treatment was associated with an increase in the relative risk of death.⁵⁴ Finally, a recent meta-analysis of observational studies demonstrated that the combination of metformin/sulfonylurea increased the relative risk of hospitalization and mortality from cardiovascular events.⁵⁵ These findings are significant as metformin/sulfonylurea is the most commonly used combination therapy in clinical practice.⁵⁶

Although the exact molecular mechanisms of metformin's glucose-lowering (and cardiovascular) effects are not entirely clear, chronic metformin treatment can impair gastrointestinal absorption of B vitamins, particularly folate.^{57,58} It has been suggested that this may lead to increased plasma homocysteine levels, leading to platelet, clotting factor, and endothelium disturbances that may contribute to the complications of atherosclerosis.²¹ Biguanides may also be contraindicated in patients with severe heart or kidney failure because of their propensity to cause lactic acidosis.⁵⁶

Troubles with Thiazolidinediones

The thiazolidinediones (TZDs) are a relatively newer class of drugs for diabetes, which make up to 25% of the oral antihyperglycemic prescriptions in clinical practice.⁵⁹ Approved TZDs such as rosiglitazone and pioglitazone⁵¹ function as insulin-sensitizing agents by acting as agonists to the gamma form of the peroxisome proliferator-activated receptor (PPAR γ), which is expressed in multiple tissues, including adipocytes.⁷ PPAR γ activation subsequently induces expression of genes involved in carbohydrate and lipid metabolism, increasing insulin sensitivity.^{51,60} Given the broader role of PPAR γ in inflammatory signaling, these agents were originally hypothesized as being capable of greater vascular protection.⁶¹

Regrettably, this early promise was diluted by studies reporting different cardiovascular problems with TZD therapy. First, TZD use leads to fluid retention and peripheral edema, with the latter manifesting clinically in about 5% of all patients.^{56,59} Increased fluid retention often causes weight gain and may worsen heart failure; hence, TZDs are contraindicated for patients with or at risk for heart failure (New York Heart Association Class III or IV).⁵⁶ Although trials and meta-analyses have revealed that both rosiglitazone and pioglitazone are associated with an increased risk of heart failure in diabetic patients,²⁸

the placebo-subtracted rate for this complication is quite low at 0.25% to 0.45% increased risk per year.^{62,63} Interestingly, a case report of two separate incidences of TZD-induced congestive heart failure has recently appeared, in which patients with no preexisting heart failure or history of cardiac disease developed cardiomyopathy after beginning TZD treatment.⁶⁴ However, findings assuming a causal link between TZD treatment and heart failure independent of fluid retention are controversial and should be interpreted with caution. A recent review has noted that type 2 diabetes patients already possess a high underlying risk of heart failure, and that TZD-associated edema may simply exacerbate or unmask preexisting cardiovascular dysfunction, as opposed to directly causing heart failure.^{60,65}

In addition to heart failure, meta-analyses have demonstrated that rosiglitazone may increase the risk of other adverse cardiovascular outcomes in type 2 diabetes patients.⁶⁶ For example, rosiglitazone may increase the relative risk of myocardial infarction, albeit by a small margin.^{67,68} Additionally, an interim report from an ongoing trial evaluating cardiac outcomes of rosiglitazone (Rosiglitazone Evaluated for Cardiac Outcomes and Regulation of Glycemia in Diabetes [RECORD] Study) has revealed that in combination with either metformin or sulfonylurea, rosiglitazone treatment is associated with a slight increase in myocardial infarction, cardiovascular hospitalization or death as compared with combination metformin/sulfonylurea treatment without rosiglitazone.²⁸ However, this study has been criticized for its design.⁶⁹ The more recent Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial showed that diabetic patients receiving intensive glucose control exhibited increased rates of cardiovascular mortality compared to patients in the standard-control group.⁷⁰ In this study, 91.2% of patients in the intensive-control group were treated with rosiglitazone compared with 57.5% of patients in the standard-control group.⁷⁰ However, according to study analyses, increased cardiovascular mortality could not be attributed to rosiglitazone treatment.⁷⁰ Some have speculated that the increased mortality in the intensive-control group may be attributable to the higher incidence and severity of hypoglycemia in this group or to the consequences of the weight gain also observed in intensively treated patients. However, the ACCORD trial found no significant relation between hypoglycemia and death or weight gain and death.⁷⁰ Finally, despite several positive metabolic effects, rosiglitazone monotherapy has also been shown to increase low-density lipoprotein cholesterol by 10% to 15%.⁷¹

Think 'Cardiovascular' when Treating Type 2 Diabetes

The Veterans Administration Diabetes Trial examined the effects of intensive glucose control using either

sulfonylurea (glimepiride), biguanide (metformin), thiazolidinediones (rosiglitazone), or insulin treatment. At the 6.25-year follow-up, no significant difference in cardiovascular outcomes was seen in the intensive control group compared with conventional treatment.^{70,72} The available agents used to treat diabetes have not been conclusively shown to reduce macrovascular disease, and, in some instances, their chronic use may promote negative cardiovascular outcomes in diabetic subjects, despite improvement in hyperglycemia. Importantly, these adverse cardiovascular side effects appear in several instances to be directly due to the mode of drug action.⁵⁶ Together, these concepts underscore the need for new drugs in diabetes that not only address blood glucose levels, but have salutary effects on the pathophysiology of CVD.

With the CVD burden attributable to diabetes increasing,⁷³ and expected to redouble over the next several decades, close and careful attention to the primary and secondary prevention strategies in this patient population is of paramount importance. Indeed, this paradigm supports the more aggressive widespread use of statins, antiplatelet agents, and antihypertensive drugs in diabetic patients, particularly those at highest risk of CVD.

Might Incretin-Targeted Therapeutics Hold Promise?

Recently, considerable interest has been generated by a novel class of antihyperglycemic agents that act at distinct levels of the “incretin” pathway. Incretins are a group of gastrointestinal hormones, predominantly GLP-1 and gastric inhibitory polypeptide, which increase postprandial insulin release from pancreatic β cells in a glucose-dependent manner. The observation that food or oral glucose administration triggers a greater insulin release compared with equal amounts of intravenously infused glucose led to the discovery of the incretin concept.⁷⁴ Gut-derived signals stimulated by oral nutrient ingestion are postulated to represent potent insulin secretagogues responsible for augmented insulin release. Here, we begin by discussing the biology of the incretin hormone GLP-1 and how it has been targeted as a therapeutic for diabetes.

GLP-1 is an Incretin

Although several gut hormones can demonstrate incretin-like effects, evidence from previous studies in humans and animal models have suggested that GLP-1 and gastric inhibitory polypeptide account for almost the entire incretin effect that facilitates disposal of ingested nutrients. GLP-1 also inhibits glucagon secretion^{75,76} and gastric emptying.⁷⁷ Acute injection of GLP-1 produces a transient reduction in food intake,⁷⁸ whereas prolonged administration of GLP-1 is associated with weight loss.^{79–82} GLP-1 analogues and

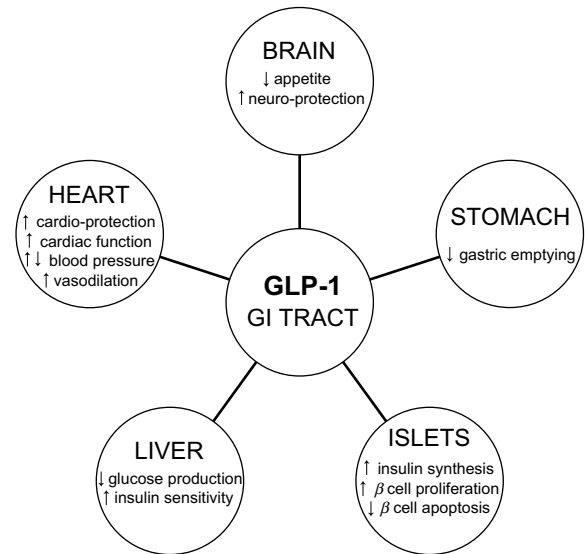


Figure 1. Physiological actions of incretins on various tissues.

GLP-1 receptor (GLP-1R) agonists also produce modest weight loss in diabetic subjects (Figure 1).^{83–85}

Of particular potential relevance to the cardiovascular field, GLP-1 exerts several other actions on pancreatic β cells besides the stimulation of insulin secretion. GLP-1 promotes insulin biosynthesis, β -cell proliferation and survival,^{86,87} and differentiation of exocrine cells or islet precursors toward a more differentiated β -cell state.^{88–90} GLP-1R–dependent augmentation of β -cell mass has been observed in diverse models,^{87,91–93} and increased functioning β -cell mass may delay the development of diabetes in rodents.^{94,95} Finally, GLP-1 reduced peroxide-induced apoptosis in insulinoma cells,⁹⁶ and attenuated cytokine-induced apoptosis of β cells.⁸⁶ Hence, GLP-1R–dependent activation of both proliferative and antiapoptotic pathways in the endocrine pancreas provides complementary mechanisms for enhancing β -cell mass.

GLP-1 Synthesis, Secretion, and Degradation

GLP-1 is derived from a proglucagon precursor that encodes GLP-1 and the related proglucagon-derived peptides: glucagon, GLP-2, oxyntomodulin, and glicentin.⁹⁷ Two forms of GLP-1 are secreted after meal ingestion, which differ by a single amino acid: GLP-1(7-37) and GLP-1(7-36) amide. Both are equipotent and exhibit identical plasma half-life and biological activities through the same receptor. However, the majority ($\sim 80\%$) of circulating active GLP-1 appears to be GLP-1(7-36) amide (the latter being referred to more commonly as GLP-1). GLP-1 is synthesized within the L cells of the intestine. Plasma levels of GLP-1 are low in the fasting state and rise rapidly within minutes of food ingestion. GLP-1 secretion from the distal gut is controlled by neural and endocrine signals

initiated by nutrient entry in the proximal gut and by direct nutrient contact with L cells.^{98,99} After secretion, GLP-1 is rapidly cleaved to GLP-1(9-36) at the position 2 alanine by the widely expressed enzyme dipeptidyl peptidase-4 (DPP-4). The half-life of exogenously infused bioactive GLP-1 is less than 3 minutes because of enzymatic inactivation and renal clearance.

The GLP-1 Receptor

The actions of GLP-1 are transduced by a 7 transmembrane-spanning G protein-coupled receptor, the GLP-1R, which is expressed in islet β cells, as well as in the heart and vasculature.¹⁰⁰ Ligand activation of the GLP-1R stimulates adenylate cyclase via *G α s*, which leads to an increase in intracellular cyclic adenosine monophosphate (cAMP) and activation of protein kinase A. Hence, GLP-1 acts directly through the cAMP-protein kinase A pathway to enhance and sensitize β cells to glucose-stimulated insulin secretion. Glucose metabolism in the β -cell causes an increase in the concentration of ATP and raises the cytoplasmic ATP to adenosine diphosphate (ADP) ratio, which then leads to depolarization of the plasma membrane following closure of

ATP-sensitive K^+ channels. This permits opening of voltage-dependent L-type Ca^{2+} channels and increases cytosolic Ca^{2+} , which triggers fusion of insulin-containing secretory vesicles to the plasma membrane. Exocytosis of insulin follows rapidly.

The physiological importance of endogenous GLP-1 has been studied intensively with specific GLP-1R antagonists. Infusion of exendin(9-39), the truncated lizard salivary gland peptide that functions as a GLP-1R antagonist in rats, mice, baboons, or humans, produces an increase in fasting glucose and glycemic excursion after glucose loading, in association with reduced levels of circulating insulin.^{101–104} Acute intracerebroventricular injection of exendin(9-39) increases food intake in satiated rats,⁷⁸ and repeated daily intracerebroventricular administration induces weight gain.⁷⁹ Comparable studies with exendin(9-39) in humans demonstrate an essential role of endogenous GLP-1 in the regulation of glucagon and insulin secretion.^{105,106}

Drugs Targeting the GLP-1–GLP-1R Axis

The rapid degradation and clearance of endogenous and exogenous GLP-1¹⁰⁷ by DPP-4 has spurred the development of degradation-resistant GLP-1 analogues capable of extended in vivo activity and DPP-4 inhibitors capable of prolonging the activity of endogenous GLP-1. Two different types of GLP-1-targeted drugs, namely a GLP-1R agonist (*exendin-4*) and a DPP-4 inhibitor (*sitagliptin*), have been approved in many countries for the treatment of type 2 diabetes, including the United States. A second

DPP-4 inhibitor, vildagliptin, is also used in many countries. Additional GLP-1 analogues and DPP-4 inhibitors are undergoing clinical trials or are being reviewed for applications as novel drugs. Collectively, if these agents are found to have beneficial effects on the pathophysiology of cardiac and vascular disease, they may emerge with a meaningful advantage over the more traditional diabetes drugs discussed previously.

Exendin-4

Exendin-4 (exenatide) is a 39 amino acid naturally occurring GLP-1R agonist isolated from the venom of the lizard *Heloderma suspectum*.¹⁰⁸ The Food and Drug Administration (FDA) approved synthetic exendin-4 for treatment of type 2 diabetes in April 2005. Exendin-4 binds to and activates the GLP-1R, but is highly resistant to the proteolytic action of DPP-4 in vivo. Therefore, exendin-4 is able to effectively enhance insulin secretion in diabetic subjects,¹⁰⁹ and daily subcutaneous injections are able to significantly reduce blood glucose and glycosylated hemoglobin,¹¹⁰ an accepted measure of blood glucose levels over time. Reported side effects of exenatide include nausea, vomiting, diarrhea, and loss of appetite and weight. Importantly, a few cases of acute pancreatitis have also been reported in patients treated with exenatide, prompting new prescribing instructions.

DPP-4 Inhibitors

Sitagliptin is an orally active DPP-4 inhibitor that prevents degradation of endogenously released GLP-1, increasing its circulating levels and prolonging its half-life. This agent has been approved for the treatment of type 2 diabetes in many different countries, including the U.S. since 2006. Clinical studies have shown that DPP-4 inhibitors reduce fasting and post-prandial blood glucose levels and glycosylated hemoglobin.^{111,112} The most common known side effects of sitagliptin include symptoms of an upper respiratory infection, such as stuffy or runny nose, sore throat, and headache.

The Cardioprotective and Vasoactive Actions of GLP-1

Studies of cardiovascular pathophysiology have primarily focused on elucidating the cardiac effects of GLP-1 in animal models of heart failure, and two pilot studies have also been conducted in humans. Among the first, conscious dogs with pacing-induced dilated cardiomyopathy received a 48-hour infusion of recombinant GLP-1 (1.5 pmol/kg/min) or saline (3 mL/d). Compared with saline-treated controls, GLP-1 infusions improved left ventricular contractility (92%), stroke volume (102%), and cardiac output (57%).¹¹³ GLP-1-treated dogs exhibited increased myocardial glucose uptake (46%) and oxygen

consumption (9.4%), suggesting enhanced oxidative phosphorylation. As such, these authors posited that the beneficial effects of GLP-1R stimulation are due primarily to modulation of myocardial metabolism.¹¹³ In a small pilot study of 10 patients undergoing angioplasty for acute myocardial infarction, continuous 72-hour infusions of recombinant human GLP-1 (1.5 pmol/kg/min) significantly improved left ventricular ejection fraction (11%) and wall motion scores (21%) compared with untreated controls.³² Interestingly, these improvements in cardiovascular function were sustained, remaining detectable in some patients even months after cessation of GLP-1 administration. The benefits of GLP-1 were independent of infarct location or history of diabetes; however, the molecular and cellular mechanisms underlying the observation were not explored.³² Similarly, a 5-week course of GLP-1 infusion improved left ventricular ejection fraction (4.4% to 7.7%; diabetics/nondiabetics) and exercise capacity (VO₂-max: 3.2 to 3.3 mL/kg/min) in a pilot study of both diabetic and nondiabetic subjects with congestive heart failure.³⁶ Finally, preoperative treatment with GLP-1 resulted in glycemic control and comparable hemodynamic recovery without high-dose insulin or inotropes following coronary artery bypass.¹¹⁴

Bose et al demonstrated that treatment with GLP-1 (4.8 pmol/kg/min in vivo; 0.3 nmol in vitro) significantly reduced infarct size¹¹⁵ and that this protective effect was blocked by the GLP-1R antagonist exendin(9-39), and by inhibitors of cAMP, phosphoinositide 3-kinases, and p42/44 mitogen activated protein kinase (MAPK) in both isolated perfused rat hearts and in a whole animal model of ischemia-reperfusion myocardial injury.¹¹⁵ Intriguingly, these data implicate *multiple* downstream signaling pathways, each of which appears critical for mediating the cardioprotective effects of GLP-1. Most importantly, the benefits of GLP-1 in perfused hearts (ie, in vitro) appear to rule out an essential role for indirect (ie, noncardiac) effects of GLP-1 on myocardial protection.

Cardioprotective and Vasodilatory Actions of GLP-1 are Partly Mediated by Glp-1r-Independent Pathways

Given the accumulating evidence supporting a cardioprotective role for GLP-1, we sought to explore the precise mechanisms underlying these beneficial effects. Our original hypothesis was that the cardioprotection endowed by GLP-1 treatment would be derived from activation of the classic GLP-1R and its putative downstream signaling pathways. Hence, we expected that the protective effects of GLP-1 would be completely absent in mice lacking a functional GLP-1R (Glp-1r^{-/-}). Having previously generated this knockout animal¹¹⁶ and used it to examine the biological actions of GLP-1 in the pancreatic islet^{117–119} and brain,¹²⁰ we next examined the importance of the GLP-1R in the

cardiovascular system.¹²¹ Our initial report on the cardiac phenotype of Glp-1r^{-/-} mice suggested that the GLP-1R played a role in normal cardiac structure and function, with Glp-1r^{-/-} mice exhibiting reduced heart rates, impaired inotropic responses, and abnormal left ventricular mass as compared with wild-type controls.¹²¹

More Lessons from Glp-1r Knockout Mice

Employing an ex vivo perfused heart preparation and an ischemia-reperfusion (I/R) injury protocol, we showed that pretreatment with GLP-1 (0.3 nM) significantly enhanced the recovery of cardiac function (as determined by left ventricular developed pressure), and decreased cardiomyocyte necrosis (as measured by lactate dehydrogenase (LDH) release) after I/R.¹⁰⁰ Interestingly, pretreatment with the GLP-1R agonist exendin-4 (5 nM) resulted in a similar degree of cardioprotection from I/R injury in hearts of wild-type mice, but required a 10-fold higher dose as compared with native GLP-1. We were particularly surprised to discover that the protective effects of native GLP-1 were preserved in mice lacking a functional GLP-1R. Together with data showing that the salutary effects of exendin-4 (5 nM) were significantly reduced but not absent in Glp-1r^{-/-} hearts, this finding strongly suggested the existence of a GLP-1R-independent signaling pathway for cardioprotection.

An Alternate GLP-1 Receptor?

The question of a second structurally and functionally distinct receptor for GLP-1 has arisen previously. Several studies have demonstrated that not all of the effects of GLP-1 can be blocked by the known GLP-1R antagonist exendin(9-39) in organs such as liver and gut. Daniel et al observed that exendin(9-39) did not block the inhibitory actions of GLP-1 on gastrointestinal motility or gastric acid secretion.¹²² Additionally, GLP-1 increased basal and acute insulin-stimulated glucose uptake as well as GLUT1 and GLUT4 protein levels in fully differentiated 3T3-L1 adipocytes, where the existence of the known GLP-1R has not been conclusively demonstrated.^{123,124}

GLP-1(9-36): A Not-So-Inactive Metabolite

We next turned our attention to examining the potential role of GLP-1(9-36), the DPP-4-generated metabolite of GLP-1, as a potentially critical intermediary in GLP-1-mediated and GLP-1R-independent protection against I/R injury. Although GLP-1(9-36) has traditionally been considered as either an inactive or weak agonist of the GLP-1R, the vast majority of previous studies examining the cardiovascular effects of GLP-1 have been performed without inhibition of the highly active DPP-4 enzyme, making it difficult to determine whether the cardiovascular

effects of GLP-1 were derived from GLP-1 itself, GLP-1(9-36), or both. Two recent reports ascribe protective cardiovascular effects directly to GLP-1(9-36). First, Nikolaidis et al observed that treatment with the truncated peptide GLP-1(9-36) increased myocardial glucose uptake and improved left ventricular performance in conscious dogs with dilated cardiomyopathy.¹²⁵ Second, Sonne et al demonstrated that administration of GLP-1(9-36) following global ischemia significantly improved left ventricular pressure, although the treatment failed to reduce infarct size.¹²⁶

In our studies examining the effects of GLP-1(9-36) in I/R, we found that unlike GLP-1, pretreatment with GLP-1(9-36) before I/R exerted no beneficial effects on cardiac function. By contrast, when GLP-1(9-36) was infused after ischemia (ie, during the reperfusion phase), it dramatically augmented functional recovery and decreased cellular injury in hearts from both wild-type and *Glp-1r^{-/-}* mice. Together, these data indicated that the beneficial effects of GLP-1 are mediated at least in part through GLP-1(9-36)-dependent and GLP-1R-independent mechanism.¹⁰⁰

GLP-1 Exhibits Vasodilatory Action

Another compelling finding of our study was that both GLP-1 and GLP-1(9-36) exhibited significant vasodilatory effects, increasing coronary flow in constant pressure-perfused isolated hearts. To further examine the vascular actions of GLP-1 and GLP-1(9-36), we tested these agents in phenylephrine precontracted isolated mesenteric arteries. Both agents significantly dilated precontracted mesenteric arteries from wild-type mice. Furthermore, the vasodilatory effects of GLP-1 and GLP-1(9-36) were maintained in *Glp-1r^{-/-}* mice, suggesting a *Glp-1r*-independent vasodilatory mechanism of GLP-1(9-36). We subsequently found these vasodilatory effects of both GLP-1 and GLP-1(9-36) to correlate with an increase in cyclic guanosine monophosphate (cGMP) release. Also, these vasodilatory actions were attenuated by preincubation of vessels with NG-nitro-L-arginine, a nonselective nitric oxide synthase (NOS) inhibitor, suggesting that at least part of their vasodilatory mechanism is nitric oxide (NO)/cGMP-dependent. Unlike GLP-1 or GLP-1(9-36), Exendin-4 did not produce any vasodilation or cGMP release.

It has been well documented that NO activates soluble guanylate cyclase, which leads to the production of cGMP and activation of protein kinase G.^{127,128} Numerous studies have suggested that the NO-cGMP-protein kinase G signaling pathway induces cardioprotection against I/R injury via the opening of the mitochondrial K_{ATP} channel. This channel is considered one of the end effectors in ischemic preconditioning, a well-known intrinsic protective mechanism against I/R injury conferred by short periods of I/R sequences before introduction of prolonged ischemia.^{127,129–131} Several independent lines of evidence also demonstrate that administration of an NO donor

such as sodium nitroprusside, natriuretic peptide, or a phosphodiesterase-5 inhibitor enhanced cardiac function or reduced infarct size in ischemic hearts through increased cGMP levels mediating vasodilation.^{129,132–134} Moreover, a recent study from Sangawa et al showed that postischemic cardiac function was improved when atrial natriuretic peptide was infused only during reperfusion.¹³⁴ Cardioprotective effects were not observed with atrial natriuretic peptide administration before the onset of ischemia, which is similar to the results we obtained with GLP-1(9-36).

To verify whether DPP-4-generated GLP-1(9-36) plays a critical role in GLP-1-mediated cardioprotection and vasodilation, we tested the effects of GLP-1 treatment in the presence of the commercially available DPP-4 inhibitor Sitagliptin. On blocking the metabolic conversion of GLP-1 to GLP-1(9-36) in precontracted mesenteric arteries and in isolated hearts undergoing I/R, we showed that both the vasodilatory and cardioprotective effects of GLP-1 were significantly reduced.¹⁰⁰ These experiments further supported that the cardiovascular effects of GLP-1 are partly mediated by a GLP-1(9-36)-dependent pathway. Importantly, significant degrees of vasodilation and cardioprotection remained after DPP-4 inhibition, supporting the notion that some of the cardiovascular effects of native GLP-1 are still mediated by GLP-1R-dependent mechanisms.

Other experiments support a vasoactive role for GLP-1. Golpon et al and Nystrom et al observed that GLP-1 caused both dose- and time-dependent relaxation of rat pulmonary artery rings and femoral arteries.^{135,136} Continuous infusion of GLP-1 also improved peripheral blood flow in short-term studies of human subjects with type 2 diabetes. In addition, both short and longer term treatments with GLP-1 receptor agonists reduced systolic and diastolic BPs in diabetic patients. These BP effects may not entirely be attributable to the indirect benefits of weight loss, because they were evident after even a few weeks of treatment.^{137–140} Most recently, Green et al demonstrated that five peptides structurally related to GLP-1, including exendin(9-39), a potent GLP-1R antagonist, caused concentration-dependent vasorelaxation. They further revealed that these relaxant effects are mediated via cAMP- K_{ATP} channel-dependent mechanisms, because the observed vasorelaxant effects were completely blunted by pharmacological blockade of either cAMP (Rp-cAMPS) or K_{ATP} channels (glybenclamide), providing evidence that GLP-1 may also modulate vascular function through classic GLP-1R-dependent adenylate cyclase-coupled mechanisms.¹⁴¹

Implications for Cardiovascular Pathophysiology

Based on accumulating evidence, we propose a novel two-pathway schema for cardiovascular actions of GLP-1: GLP-1-initiated classic GLP-1R activation that results in ischemic “preconditioning” and vasodilatory actions; and

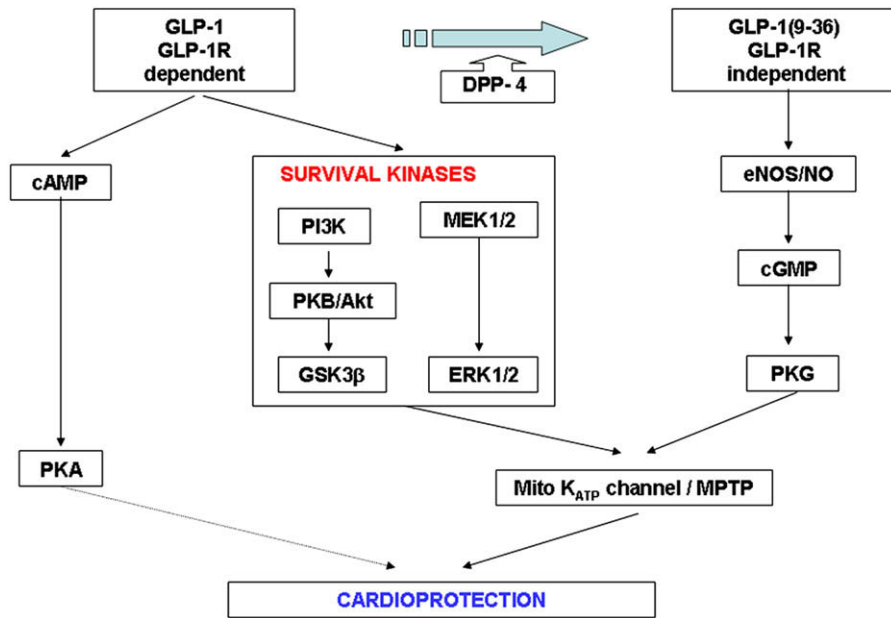


Figure 2. Putative cardioprotective mechanisms of GLP-1 receptor agonists. cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; DPP-4, dipeptidyl peptidase-4; eNOS, endothelial nitric oxide synthase; ERK1/2, extracellular signal-regulated kinases; GLP-1R, glucagon-like peptide-1 receptor; GSK3 β , glycogen synthase kinase 3 beta; MEK1/2, mitogen-activated protein kinase; Mito K_{ATP} channel, mitochondrial adenosine triphosphate-dependent potassium channel; MPTP, mitochondrial permeability transition pores; NO, nitric oxide; PI3K, phosphoinositide 3-kinases; PKA, protein kinase A; PKB/Akt, protein kinase B; PKG, protein kinase G.

GLP-1(9-36)–dependent effects via a putative alternate receptor of ischemic “postconditioning,” and vasodilatation through a NO/cGMP-dependent mechanism, which are both known to participate in cardioprotection in the setting of I/R injury (Figure 2).^{129,132}

Conclusions

In December 2008, the FDA issued guidance that all new drugs developed for the treatment of type 2 diabetes should be subjected to study to rule out any increase in the risk of cardiovascular events. A case can be made that such safety information should also be sought for drugs already on the market, because for the majority of traditional oral agents used to control hyperglycemia in diabetic patients, long-term effects on cardiovascular outcomes are either undetermined, negative, or, at best, neutral. Because diabetes is closely linked to CVD, we propose that a careful (re)examination of the mechanisms of action of the traditional anti-diabetic drugs on cardiovascular physiology be encouraged. Indeed, our overview of the current literature in this field suggests that conventional oral diabetic treatments present diverse cardiovascular effects. By contrast, preclinical data for a new class of incretin-based diabetic treatments such as GLP-1, its derivatives, and inhibitors of its degradation enzyme DPP-4, suggest that these agents may enable cardioprotective and vasodilatory effects in addition to glycemic control. Notwithstanding the many favorable cardiovascular effects of GLP-1/incretins reported in a number of studies, including ours, many questions remain unanswered. First, the number of studies directly examining the effects of GLP-1 on cardiovascular endpoints in humans has been very limited. There remains the possibility that the salutary cardiovascular effects of incretin-based therapies

observed in these small pilot human studies (and in the many more animal studies) will not translate to larger and longer term clinical studies. Furthermore, it remains uncertain as to whether the cardiovascular actions of GLP-1 observed in animal models and humans are mediated by the canonical GLP-1 receptor. It appears that GLP-1(9-36) amide, the metabolite of GLP-1, shares many of the beneficial effects of GLP-1, and may mediate these effects through an alternate receptor. Whether the latter is accessible to GLP-1 or traditional GLP-1 receptor agonists is not known. Moreover, whether the biology of GLP-1(9-36) observed in animal models hold true in humans has not been explored. As such, further studies examining the signaling pathways activated by GLP-1(9-36) and ultimate identification of its putative receptor are also needed.

Finally, we believe there is compelling need for carefully designed clinical-experimental studies specifically investigating the cardiovascular impact of GLP-1/incretin-based therapies in diabetic patients. Although preliminary studies examining the cardiovascular actions of GLP-1 have been conducted in small numbers of nondiabetic patients, larger and longer term clinical studies employing GLP-1 receptor agonists or DPP-4 inhibitors in patients with diabetes have been missing. For example, the recently launched TECOS’ trial, the large-scale randomized placebo-controlled clinical Trial to Evaluate Cardiovascular Outcomes after treatment with Sitagliptin in patients with type 2 diabetes mellitus and inadequate glycemic control on mono- or dual combination oral antihyperglycemic therapy, is an important initiative. Based on the early and predominantly preclinical findings reviewed here, together with the reduction in BP observed in human studies of GLP-1R agonists, we would predict that GLP-1–targeted therapies for diabetes may be accompanied by improved cardiovascular outcomes in

larger and longer term clinical studies. We believe that the preclinical evidence in support of this hypothesis is compelling.

References

- Rother KI. Diabetes treatment—bridging the divide. *N Engl J Med* 2007;12:1499–501.
- Grobbee DE. How to ADVANCE prevention of cardiovascular complications in type 2 diabetes. *Metab Clin Exp* 2003;52:24–8.
- Cowie CC, Rust KF, Byrd-Holt DD, Eberhardt MS, Flegal KM, Engelgau MM, et al. Prevalence of diabetes and impaired fasting glucose in adults in the U.S. population: National Health and Nutrition Examination Survey 1999–2002. *Diabetes Care* 2006;29:1263–8.
- Milicevic Z, Raz I, Beattie SD, Campaigne BN, Sarwat S, Gromniak E, et al. Natural history of cardiovascular disease in patients with diabetes: role of hyperglycemia. *Diabetes Care* 2008;31:S155–60.
- Laing SP, Swerdlow AJ, Slater SD, Burden AC, Morris A, Waugh NR, et al. Mortality from heart disease in a cohort of 23,000 patients with insulin-treated diabetes. *Diabetologia* 2003;46:760–5.
- Retnakaran R, Zinman B. Type 1 diabetes, hyperglycaemia, and the heart. *Lancet* 2008;371:1790–9.
- Stolar MW, Chilton RJ. Type 2 diabetes, cardiovascular risk, and the link to insulin resistance. *Clin Ther* 2003;25:B4–31.
- Stamler J, Vaccaro O, Neaton JD, Wentworth D. Diabetes, other risk factors, and 12-year cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabetes Care* 1993;16:434–44.
- Haffner SM, Lehto S, Ronnema T, Pyorala K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med* 1998;339:229–34.
- Laakso M. Hyperglycemia as a risk factor for cardiovascular disease in type 2 diabetes. *Prim Care* 1999;26:829–39.
- Haffner SM. Impaired glucose tolerance, insulin resistance, and cardiovascular disease. *Diabetes Med* 1997;14:S12–8.
- Gerstein HC. Glucose: a continuous risk factor for cardiovascular disease. *Diabetes Med* 1997;14:S25–31.
- Mazzone T, Chait A, Plutzky J. Cardiovascular disease risk in type 2 diabetes mellitus: insights from mechanistic studies. *Lancet* 2008;371:1800–9.
- Levy D, Larson MG, Vasan RS, Kannel WB, Ho KK. The progression from hypertension to congestive heart failure. *JAMA* 1996;275:1557–62.
- Kaplan NM, Opie LH. Controversies in hypertension. *Lancet* 2006;367:168–76.
- Freis ED, Papademetriou V. Current drug treatment and treatment patterns with antihypertensive drugs. *Drugs* 1996;52:1–16.
- Julius S. Coronary disease in hypertension: a new mosaic. *J Hypertens Suppl* 1997;15:S3–10.
- Randomised trial of cholesterol lowering in 4,444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* 1994;344:1383–9.
- Lacy CR, Suh DC, Barone JA, Bueno M, Moylan D, Swartz C, et al. Impact of a targeted intervention on lipid-lowering therapy in patients with coronary artery disease in the hospital setting. *Arch Intern Med* 2002;162:468–73.
- Aronow HD, Topol EJ, Roe MT, Houghtaling PL, Wolski KE, Lincoff AM, et al. Effect of lipid-lowering therapy on early mortality after acute coronary syndromes: an observational study. *Lancet* 2001;357:1063–8.
- Fisman EZ, Motro M, Tenenbaum A. Non-insulin anti-diabetic therapy in cardiac patients: current problems and future prospects. *Adv Cardiol* 2008;45:154–70.
- Uwaifo GI, Ratner RE. Differential effects of oral hypoglycemic agents on glucose control and cardiovascular risk. *Am J Cardiol* 2007;99:51B–567.
- Brady PA, Al-Suwaidi J, Kopecky SL, Terzic A. Sulfonylureas and mortality in diabetic patients after myocardial infarction. *Circulation* 1998;97:709–10.
- Calvert JW, Gundewar S, Jha S, Greer JJ, Bestermann WH, Tian R, et al. Acute metformin therapy confers cardioprotection against myocardial infarction via AMPK-eNOS-mediated signaling. *Diabetes* 2008;57:696–705.
- Couzin J. Drug safety. Heart attack risk overshadows a popular diabetes therapy. *Science* 2007;316:1550–1.
- Davis BJ, Xie Z, Viollet B, Zou MH. Activation of the AMP-activated kinase by anti-diabetes drug metformin stimulates nitric oxide synthesis in vivo by promoting the association of heat shock protein 90 and endothelial nitric oxide synthase. *Diabetes* 2006;55:496–505.
- Garratt KN, Brady PA, Hassinger NL, Grill DE, Terzic A, Holmes DR Jr. Sulfonylurea drugs increase early mortality in patients with diabetes mellitus after direct angioplasty for acute myocardial infarction. *J Am Coll Cardiol* 1999;33:119–24.
- Home PD, Pocock SJ, Beck-Nielsen H, Gomis R, Hanefeld M, Jones NP, et al. Rosiglitazone evaluated for cardiovascular outcomes—an interim analysis. *N Engl J Med* 2007;357:28–38.
- Lautamaki R, Airaksinen KE, Seppanen M, Toikka J, Luotolahti M, Ball E, et al. Rosiglitazone improves

- myocardial glucose uptake in patients with type 2 diabetes and coronary artery disease: a 16-week randomized, double-blind, placebo-controlled study. *Diabetes* 2005;54:2787–94.
30. Liao Y, Takashima S, Zhao H, Asano Y, Shintani Y, Minamino T, et al. Control of plasma glucose with alpha-glucosidase inhibitor attenuates oxidative stress and slows the progression of heart failure in mice. *Cardiovasc Res* 2006;70:107–16.
 31. Libby P, Maroko PR, Braunwald E. The effect of hypoglycemia on myocardial ischemic injury during acute experimental coronary artery occlusion. *Circulation* 1975;51:621–6.
 32. Nikolaidis LA, Mankad S, Sokos GG, Miske G, Shah A, Elahi D, et al. Effects of glucagon-like peptide-1 in patients with acute myocardial infarction and left ventricular dysfunction after successful reperfusion. *Circulation* 2004;109:962–5.
 33. Olsson J, Lindberg G, Gottsater M, Lindwall K, Sjostrand A, Tisell A, et al. Increased mortality in type 2 diabetic patients using sulphonylurea and metformin in combination: a population-based observational study. *Diabetologia* 2000;43:558–60.
 34. Quast U, Stephan D, Bieger S, Russ U. The impact of ATP-sensitive K⁺ channel subtype selectivity of insulin secretagogues for the coronary vasculature and the myocardium. *Diabetes* 2004;53:S156–64.
 35. Sack MN, Yellon DM. Insulin therapy as an adjunct to reperfusion after acute coronary ischemia: a proposed direct myocardial cell survival effect independent of metabolic modulation. *J Am Coll Cardiol* 2003;41:1404–7.
 36. Sokos GG, Nikolaidis LA, Mankad S, Elahi D, Shannon RP. Glucagon-like peptide-1 infusion improves left ventricular ejection fraction and functional status in patients with chronic heart failure. *J Card Fail* 2006;12:694–9.
 37. Krentz AJ, Bailey CJ. Oral anti-diabetic agents: current role in type 2 diabetes mellitus. *Drugs* 2005;65:385–411.
 38. Ashcroft FM. Mechanisms of the glycaemic effects of sulphonylureas. *Horm Metab Res* 1996;28:456–63.
 39. Kramer W, Muller G, Geisen K. Characterization of the molecular mode of action of the sulphonylurea, glibenclamide, at beta cells. *Horm Metab Res* 1996;28:464–8.
 40. Evans JM, Ogston SA, Emslie-Smith A, Morris AD. Risk of mortality and adverse cardiovascular outcomes in type 2 diabetes: a comparison of patients treated with sulphonylureas and metformin. *Diabetologia* 2006;49:930–6.
 41. Meier JJ, Deifuss S, Klamann A, Schmiegel W, Nauck MA. Influence of an anti-diabetic treatment with sulphonylurea drugs on long-term survival after acute myocardial infarction in patients with type 2 diabetes. The LANGendreer Myocardial infarction and Blood glucose in Diabetic patients Assessment (LAMBDA). *Exp Clin Endocrinol Diabetes* 2003;111:344–50.
 42. Gross GJ, Auchampach JA. Role of ATP-dependent potassium channels in myocardial ischaemia. *Cardiovasc Res* 1992;26:1011–6.
 43. Lazdunski M. Ion channel effects of anti-diabetic sulphonylureas. *Horm Metab Res* 1996;28:488–95.
 44. Meier JJ, Gallwitz B, Schmidt WE, Mugge A, Nauck MA. Is impairment of ischaemic preconditioning by sulphonylurea drugs clinically important? *Heart (British Cardiac Society)* 2004;90:9–12.
 45. Gribble FM, Reimann F. Pharmacological modulation of K(ATP) channels. *Biochem Soc Trans* 2002;30:333–9.
 46. Cole WC, McPherson CD, Sontag D. ATP-regulated K⁺ channels protect the myocardium against ischemia/reperfusion damage. *Circ Res* 1991;69:571–81.
 47. Cleveland JC Jr, Meldrum DR, Cain BS, Banerjee A, Harken AH. Oral sulphonylurea hypoglycemic agents prevent ischemic preconditioning in human myocardium. Two paradoxes revisited. *Circulation* 1997;96:29–32.
 48. Toombs CF, McGee S, Johnston WE, Vinten-Johansen J. Myocardial protective effects of adenosine. Infarct size reduction with pretreatment and continued receptor stimulation during ischemia. *Circulation* 1992;86:986–94.
 49. Patel A, MacMahon S, Chalmers J, Neal B, Billot L, Woodward M, et al. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *N Engl J Med* 2008;358:2560–72.
 50. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med* 2008;359:1577–89.
 51. Cheng AY, Fantus IG. Oral antihyperglycemic therapy for type 2 diabetes mellitus. *CMAJ* 2005;172:213–26.
 52. Sasali A, Leahy JL. Is metformin cardioprotective? *Diabetes Care* 2003;26:243–4.
 53. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998;352:854–65.
 54. Fisman EZ, Tenenbaum A, Benderly M, Goldbourt U, Behar S, Motro M. Antihyperglycemic treatment in diabetics with coronary disease: increased metformin-associated mortality over a 5-year follow-up. *Cardiology* 1999;91:195–202.
 55. Rao AD, Kuhadiya N, Reynolds K, Fonseca VA. Is the combination of sulphonylureas and metformin associated with an increased risk of cardiovascular disease or all-cause mortality? A meta-analysis of observational studies. *Diabetes Care* 2008;31:1672–8.

56. Fisman EZ, Tenenbaum A, Motro M, Adler Y. Oral anti-diabetic therapy in patients with heart disease. A cardiologic standpoint. *Herz* 2004;29:290–8.
57. Lee AJ. Metformin in noninsulin-dependent diabetes mellitus. *Pharmacotherapy* 1996;16:327–51.
58. Adams JF, Clark JS, Ireland JT, Kesson CM, Watson WS. Malabsorption of vitamin B12 and intrinsic factor secretion during biguanide therapy. *Diabetologia* 1983;24:16–8.
59. McGuire DK, Inzucchi SE. New drugs for the treatment of diabetes mellitus: part I: thiazolidinediones and their evolving cardiovascular implications. *Circulation* 2008;117:440–9.
60. Irons BK, Greene RS, Mazzolini TA, Edwards KL, Sleeper RB. Implications of rosiglitazone and pioglitazone on cardiovascular risk in patients with type 2 diabetes mellitus. *Pharmacotherapy* 2006;26:168–81.
61. Martens FM, Rabelink TJ, Op't Roodt J, de Koning EJ, Visseren FL. TNF-alpha induces endothelial dysfunction in diabetic adults, an effect reversible by the PPAR-gamma agonist pioglitazone. *Eur Heart J* 2006;27:1605–9.
62. Rohatgi A, McGuire DK. Effects of the thiazolidinedione medications on micro- and macrovascular complications in patients with diabetes-update 2008. *Cardiovasc Drugs Ther* 2008;22:233–40.
63. Dormandy JA, Charbonnel B, Eckland DJ, Erdmann E, Massi-Benedetti M, Moules IK, et al. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. *Lancet* 2005;366:1279–89.
64. Cheng AY, Fantus IG. Thiazolidinedione-induced congestive heart failure. *Ann Pharmacother* 2004;38:817–20.
65. Erdmann E, Wilcox RG. Weighing up the cardiovascular benefits of thiazolidinedione therapy: the impact of increased risk of heart failure. *Eur Heart J* 2008;29:12–20.
66. Sarafidis PA. Thiazolidinedione derivatives in diabetes and cardiovascular disease: an update. *Fundam Clin Pharmacol* 2008;22:247–64.
67. Khanderia U, Pop-Busui R, Eagle KA. Thiazolidinediones in type 2 diabetes: a cardiology perspective. *Ann Pharmacother* 2008;42:1466–74.
68. Nissen SE, Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *N Engl J Med* 2007;356:2457–71.
69. Psaty BM, Furberg CD. The record on rosiglitazone and the risk of myocardial infarction. *N Engl J Med* 2007;357:67–9.
70. Hoogwerf BJ. Does intensive therapy of type 2 diabetes help or harm? Seeking accord on ACCORD. *Cleve Clin J Med* 2008;75:729–37.
71. DeFronzo RA. Pharmacologic therapy for type 2 diabetes mellitus. *Ann Intern Med* 1999;131:281–303.
72. Gore MO, McGuire DK. The 10-year post-trial follow-up of the United Kingdom Prospective Diabetes Study (UKPDS): cardiovascular observations in context. *Diab Vasc Dis Res* 2009;6:53–5.
73. Fox CS, Coady S, Sorlie PD, D'Agostino RB Sr, Pencina MJ, Vasan RS, et al. Increasing cardiovascular disease burden due to diabetes mellitus: the Framingham Heart Study. *Circulation* 2007;115:1544–50.
74. Elrick H, Stimmler L, Hlad CJ, Arai Y. Plasma insulin responses to oral and intravenous glucose administration. *J Clin Endocrinol Metab* 1964;24:1076–82.
75. Matsuyama T, Komatsu R, Namba M, Watanabe N, Itoh H, Tarui S. Glucagon-like peptide-1 (7–36 amide): a potent glucagonostatic and insulinotropic hormone. *Diabetes Res Clin Pract* 1988;5:281–4.
76. Weir GC, Mojsos S, Hendrick GK, Habener JF. Glucagon-like peptide-1-(7–37) actions on endocrine pancreas. *Diabetes* 1989;38:338–42.
77. Wettergren A, Schjoldager B, Mortensen PE, Myhre J, Christiansen J, Holst JJ. Truncated GLP-1 (proglucagon 78–107-amide) inhibits gastric and pancreatic functions in man. *Dig Dis Sci* 1993;38:665–73.
78. Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CMB, Meeran K, et al. A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* 1996;379:69–72.
79. Meeran K, O'Shea D, Edwards CM, Turton MD, Heath MM, Gunn I, et al. Repeated intracerebroventricular administration of glucagon-like peptide-1-(7–36) amide or exendin-(9–39) alters body weight in the rat. *Endocrinology* 1999;140:244–50.
80. Davis HR Jr, Mullins DE, Pines JM, Hoos LM, France CF, Compton DS, et al. Effect of chronic central administration of glucagon-like peptide-1 (7–36) amide on food consumption and body weight in normal and obese rats. *Obes Res* 1998;6:147–56.
81. Szayna M, Doyle ME, Betkey JA, Holloway HW, Spencer RG, Greig NH, et al. Exendin-4 decelerates food intake, weight gain, and fat deposition in Zucker rats. *Endocrinology* 2000;141:1936–41.
82. Larsen PJ, Fledelius C, Knudsen LB, Tang-Christensen M. Systemic administration of the long-acting GLP-1 derivative NN2211 induces lasting and reversible weight loss in both normal and obese rats. *Diabetes* 2001;50:2530–9.
83. Buse JB, Henry RR, Han J, Kim DD, Fineman MS, Baron AD. Effects of exenatide (exendin-4) on glycemic control over 30 weeks in sulfonylurea-treated patients with type 2 diabetes. *Diabetes Care* 2004;27:2628–35.
84. Kendall DM, Riddle MC, Rosenstock J, Zhuang D, Kim DD, Fineman MS, et al. Effects of exenatide (exendin-4) on glycemic control over 30 weeks in patients with type 2 diabetes treated with metformin and a sulfonylurea. *Diabetes Care* 2005;28:1083–91.

85. DeFronzo RA, Ratner RE, Han J, Kim DD, Fineman MS, Baron AD. Effects of exenatide (exendin-4) on glycemic control and weight over 30 weeks in metformin-treated patients with type 2 diabetes. *Diabetes Care* 2005;28:1092–100.
86. Li Y, Hansotia T, Yusta B, Ris F, Halban PA, Drucker DJ. Glucagon-like peptide-1 receptor signaling modulates beta cell apoptosis. *J Biol Chem* 2003;278:471–8.
87. Xu G, Stoffers DA, Habener JF, Bonner-Weir S. Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rats. *Diabetes* 1999;48:2270–6.
88. Abraham EJ, Leech CA, Lin JC, Zulewski H, Habener JF. Insulinotropic hormone glucagon-like peptide-1 differentiation of human pancreatic islet-derived progenitor cells into insulin-producing cells. *Endocrinology* 2002;143:3152–61.
89. Hardikar AA, Wang XY, Williams LJ, Kwok J, Wong R, Yao M, et al. Functional maturation of fetal porcine beta-cells by glucagon-like peptide-1 and cholecystokinin. *Endocrinology* 2002;143:3505–14.
90. Movassat J, Beattie GM, Lopez AD, Hayek A. Exendin 4 up-regulates expression of PDX1 and hastens differentiation and maturation of human fetal pancreatic cells. *J Clin Endocrinol Metab* 2002;87:4775–81.
91. Tourrel C, Bailbe D, Meile M-J, Kergoat M, Portha B. Glucagon-like peptide-1 and exendin-4 stimulate β -cell neogenesis in streptozotocin-treated newborn rats resulting in persistently improved glucose homeostasis at adult age. *Diabetes* 2001;50:1562–70.
92. Perfetti R, Zhou J, Doyle ME, Egan JM. Glucagon-like peptide-1 induces cell proliferation and pancreatic-duodenum homeobox-1 expression and increases endocrine cell mass in the pancreas of old, glucose-intolerant rats. *Endocrinology* 2000;141:4600–5.
93. Stoffers DA, Desai BM, DeLeon DD, Simmons RA. Neonatal exendin-4 prevents the development of diabetes in the intrauterine growth retarded rat. *Diabetes* 2003;52:734–40.
94. Wang Q, Brubaker PL. Glucagon-like peptide-1 treatment delays the onset of diabetes in 8-week-old db/db mice. *Diabetologia* 2002;45:1263–73.
95. Tourrel C, Bailbe D, Lacorne M, Meile MJ, Kergoat M, Portha B. Persistent improvement of type 2 diabetes in the Goto-Kakizaki rat model by expansion of the beta-cell mass during the prediabetic period with glucagon-like peptide-1 or exendin-4. *Diabetes* 2002;51:1443–52.
96. Hui H, Nourparvar A, Zhao X, Perfetti R. Glucagon-like peptide-1 inhibits apoptosis of insulin-secreting cells via a cyclic 5'-adenosine monophosphate-dependent protein kinase A- and a phosphatidylinositol 3-kinase-dependent pathway. *Endocrinology* 2003;144:1444–55.
97. Bell GI, Sanchez-Pescador R, Laybourn PJ, Najarian RC. Exon duplication and divergence in the human preproglucagon gene. *Nature* 1983;304:368–71.
98. Rocca AS, Brubaker PL. Role of the vagus nerve in mediating proximal nutrient-induced glucagon-like peptide-1 secretion. *Endocrinology* 1999;140:1687–94.
99. Anini Y, Hansotia T, Brubaker PL. Muscarinic receptors control postprandial release of glucagon-like peptide-1: in vivo and in vitro studies in rats. *Endocrinology* 2002;143:2420–6.
100. Ban K, Noyan-Ashraf MH, Hoefler J, Bolz SS, Drucker DJ, Husain M. Cardioprotective and vasodilatory actions of glucagon-like peptide-1 receptor are mediated through both glucagon-like peptide-1 receptor-dependent and -independent pathways. *Circulation* 2008;117:2340–50.
101. Baggio L, Kieffer TJ, Drucker DJ. Glucagon-like peptide-1, but not glucose-dependent insulinotropic peptide, regulates fasting glycemia and nonenteral glucose clearance in mice. *Endocrinology* 2000;141:3703–9.
102. Kolligs F, Fehmann H-C, Goke R, Goke B. Reduction of the incretin effect in rats by the glucagon-like peptide-1 receptor antagonist exendin(9–39)amide. *Diabetes* 1995;44:16–9.
103. Wang Z, Wang RM, Owji AA, Smith DM, Ghatei MA, Bloom SR. Glucagon-like peptide-1 is a physiological incretin in rat. *J Clin Invest* 1995;95:417–21.
104. D'alessio DA, Vogel R, Prigeon R, Laschansky E, Koerker D, Eng J, et al. Elimination of the action of glucagon-like peptide-1 causes an impairment of glucose tolerance after nutrient ingestion by healthy baboons. *J Clin Invest* 1996;97:133–8.
105. Schirra J, Sturm K, Leicht P, Arnold R, Goke B, Katschinski M. exendin(9-39)amide is an antagonist of glucagon-like peptide-1(7-36)amide in humans. *J Clin Invest* 1998;101:1421–30.
106. Edwards CM, Todd JF, Mahmoudi M, Wang Z, Wang RM, Ghatei MA, et al. Glucagon-like peptide-1 has a physiological role in the control of postprandial glucose in humans: studies with the antagonist exendin(9-39). *Diabetes* 1999;48:86–93.
107. Deacon CF, Johnsen AH, Holst JJ. Degradation of glucagon-like peptide-1 by human plasma in vitro yields an N-terminally truncated peptide that is a major endogenous metabolite in vivo. *J Clin Endocrinol Metab* 1995;80:952–7.
108. Eng J, Kleinman WA, Singh L, Singh G, Raufman JP. Isolation and characterization of exendin-4, an exendin-3 analogue from *Heloderma suspectum* venom. *J Biol Chem* 1992;267:7402–5.

109. Egan JM, Clocquet AR, Elahi D. The insulinotropic effect of acute exendin-4 administered to humans: comparison of nondiabetic state to type 2 diabetes. *J Clin Endocrinol Metab* 2002;87:1282–90.
110. Egan JM, Meneilly GS, Elahi D. Effects of one month bolus subcutaneous administration of exendin-4 in type 2 diabetes. *Am J Physiol Endocrinol Metab* 2003;284:E1072–9.
111. Garber AJ, Sharma MD. Update: vildagliptin for the treatment of type 2 diabetes. *Expert Opin Investig Drugs* 2008;17:105–13.
112. Karasik A, Aschner P, Katzeff H, Davies MJ, Stein PP. Sitagliptin, a DPP-4 inhibitor for the treatment of patients with type 2 diabetes: a review of recent clinical trials. *Curr Med Res Opin* 2008;24:489–96.
113. Nikolaidis LA, Elahi D, Hentosz T, Doverspike A, Huerbin R, Zourelis L, et al. Recombinant glucagon-like peptide-1 increases myocardial glucose uptake and improves left ventricular performance in conscious dogs with pacing-induced dilated cardiomyopathy. *Circulation* 2004;110:955–61.
114. Sokos GG, Bolukoglu H, German J, Hentosz T, Magovern GJ Jr, Maher TD, et al. Effect of glucagon-like peptide-1 (GLP-1) on glycemic control and left ventricular function in patients undergoing coronary artery bypass grafting. *Am J Cardiol* 2007;100:824–9.
115. Bose AK, Mocanu MM, Carr RD, Brand CL, Yellon DM. Glucagon-like peptide-1 (GLP-1) can directly protect the heart against ischemia/reperfusion injury. *Diabetes* 2005;54:146–51.
116. Scrocchi LA, Brown TJ, MacClusky N, Brubaker PL, Auerbach AB, Joyner AL, et al. Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide-1 receptor gene. *Nat Med* 1996;2:1254–8.
117. Hansotia T, Baggio LL, Delmeire D, Hinke SA, Yamada Y, Tsukiyama K, et al. Double incretin receptor knockout (DIRKO) mice reveal an essential role for the enteroinsular axis in transducing the glucoregulatory actions of DPP-4 inhibitors. *Diabetes* 2004;53:1326–35.
118. De Leon DD, Deng S, Madani R, Ahima RS, Drucker DJ, Stoffers DA. Role of endogenous glucagon-like peptide-1 in islet regeneration after partial pancreatectomy. *Diabetes* 2003;52:365–71.
119. Preitner F, Ibberson M, Franklin I, Binnert C, Pende M, Gjinovci A, et al. Gluco-incretins control insulin secretion at multiple levels as revealed in mice lacking GLP-1 and GIP receptors. *J Clin Invest* 2004;113:635–45.
120. Yamamoto H, Lee CE, Marcus JN, Williams TD, Overton JM, Lopez ME, et al. Glucagon-like peptide-1 receptor stimulation increases blood pressure and heart rate and activates autonomic regulatory neurons. *J Clin Invest* 2002;110:43–52.
121. Gros R, You X, Baggio LL, Kabir MG, Sadi AM, Mungro IN, et al. Cardiac function in mice lacking the glucagon-like peptide-1 receptor. *Endocrinology* 2003;144:2242–52.
122. Daniel EE, Anvari M, Fox-Threlkeld JET, McDonald TJ. Local, exendin-(9–39)-insensitive, site of action of GLP-1 in canine ileum. *Am J Physiol Gastrointest Liver Physiol* 2002;283:G595–602.
123. Wang Y, Kole HK, Montrose-Rafizadeh C, Perfetti R, Bernier M, Egan JM. Regulation of glucose transporters and hexose uptake in 3T3-L1 adipocytes: glucagon-like peptide-1 and insulin interactions. *J Mol Endocrinol* 1997;19:241–8.
124. Montrose-Rafizadeh C, Yang H, Rodgers BD, Beday A, Pritchette LA, Eng J. High potency antagonists of the pancreatic glucagon-like peptide-1 receptor. *J Biol Chem* 1997;272:21201–6.
125. Nikolaidis LA, Elahi D, Shen YT, Shannon RP. Active metabolite of GLP-1 mediates myocardial glucose uptake and improves left ventricular performance in conscious dogs with dilated cardiomyopathy. *Am J Physiol Heart Circ Physiol* 2005;289:H2401–8.
126. Sonne DP, Engstrom T, Treiman M. Protective effects of GLP-1 analogues exendin-4 and GLP-1(9–36) amide against ischemia-reperfusion injury in rat heart. *Regul Pept* 2008;146:243–9.
127. Taimor G, Hofstaetter B, Piper HM. Apoptosis induction by nitric oxide in adult cardiomyocytes via cGMP-signaling and its impairment after simulated ischemia. *Cardiovasc Res* 2000;45:588–94.
128. Cuong DV, Kim N, Youm JB, Joo H, Warda M, Lee JW, et al. Nitric oxide-cGMP-protein kinase G signaling pathway induces anoxic preconditioning through activation of ATP-sensitive K⁺ channels in rat hearts. *Am J Physiol Heart Circ Physiol* 2006;290:H1808–17.
129. Brunner F, Maier R, Andrew P, Wolkart G, Zechner R, Mayer B. Attenuation of myocardial ischemia/reperfusion injury in mice with myocyte-specific overexpression of endothelial nitric oxide synthase. *Cardiovasc Res* 2003;57:55–62.
130. D'Souza SP, Yellon DM, Martin C, Schulz R, Heusch G, Onody A, et al. B-type natriuretic peptide limits infarct size in rat isolated hearts via KATP channel opening. *Am J Physiol Heart Circ Physiol* 2003;284:H1592–600.
131. Yang XM, Philipp S, Downey JM, Cohen MV. Atrial natriuretic peptide administered just prior to reperfusion limits infarction in rabbit hearts. *Basic Res Cardiol* 2006;101:311–8.
132. Kloner RA. Cardiovascular effects of the 3 phosphodiesterase-5 inhibitors approved for the treatment of erectile dysfunction. *Circulation* 2004;110:3149–55.
133. Du Toit EF, Meiring J, Opie LH. Relation of cyclic nucleotide ratios to ischemic and reperfusion injury

- in nitric oxide-donor treated rat hearts. *J Cardiovasc Pharmacol* 2001;38:529–38.
134. Sangawa K, Nakanishi K, Ishino K, Inoue M, Kawada M, Sano S. Atrial natriuretic peptide protects against ischemia-reperfusion injury in the isolated rat heart. *Ann Thorac Surg* 2004;77:233–7.
135. Golpon HA, Puechner A, Welte T, Wichert PV, Feddersen CO. Vasorelaxant effect of glucagon-like peptide-(7–36)amide and amylin on the pulmonary circulation of the rat. *Regul Pept* 2001;102:81–6.
136. Nystrom T, Gonon AT, Sjöholm A, Pernow J. Glucagon-like peptide-1 relaxes rat conduit arteries via an endothelium-independent mechanism. *Regul Pept* 2005;125:173–7.
137. Vilsboll T. Liraglutide: a once-daily GLP-1 analogue for the treatment of type 2 diabetes mellitus. *Expert Opin Investig Drugs* 2007;16:231–7.
138. Klonoff DC, Buse JB, Nielsen LL, Guan X, Bowlus CL, Holcombe JH, et al. Exenatide effects on diabetes, obesity, cardiovascular risk factors and hepatic biomarkers in patients with type 2 diabetes treated for at least 3 years. *Curr Med Res Opin* 2008;24:275–86.
139. Amori RE, Lau J, Pittas AG. Efficacy and safety of incretin therapy in type 2 diabetes: systematic review and meta-analysis. *JAMA* 2007;298:194–206.
140. Drucker DJ, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet* 2006;36:1696–705.
141. Green BD, Hand KV, Dougan JE, McDonnell BM, Cassidy RS, Grieve DJ. GLP-1 and related peptides cause concentration-dependent relaxation of rat aorta through a pathway involving K(ATP) and cAMP. *Arch Biochem Biophys* 2008;478:136–42.

Receive Tables of Contents by e-mail

To receive Tables of Contents by e-mail, sign up through our website at www.ashjournal.com

Instructions

Log on to www.ashjournal.com and click on the “Add Table of Contents Alert” link in the Receive Free E-mail Alerts section of the home page. Complete the registration process. You will receive an e-mail message confirming that you have been added to the mailing list. Note that the Table of Contents e-mails will be sent when a new issue is posted to the Web.