

Review Article

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

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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 (DPP4) 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;■(■):1–15. © 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

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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

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106 global prevalence of diabetes,² it is estimated that more
 107 than 9% of the general U.S. population has diabetes,³
 108 with another 26% exhibiting impaired glucose tolerance.
 109 As such, a staggering 35% of Americans manifest impaired
 110 glucose homeostasis. Given the adverse consequences of
 111 hyperglycemia on health, these prevalence rates attest to
 112 a significant and worsening public health issue, burdening
 113 health care resources in both the developed and developing
 114 world.

115 116 *Diabetes and Cardiovascular Disease* 117

118 Patients with diabetes are at increased risk for cardiovas-
 119 cular disease (CVD). Indeed, CVD is the predominant
 120 cause of death in diabetic patients,⁴ with complications
 121 such as myocardial infarction and stroke causing 75% of
 122 all deaths.² Compared with nondiabetic controls, diabetic
 123 patients have a several-fold increased risk of developing
 124 CVD.⁵ Type 1 patients possess a 10 times higher risk
 125 than the general population,⁶ whereas type 2 patients
 126 have a 3 times higher risk of cardiovascular death.⁷ In
 127 general, diabetic patients have a 3 to 5 times increased inci-
 128 dence of death from coronary heart disease compared with
 129 nondiabetic subjects, a risk level that equates with nondia-
 130 betic patients who have already suffered a myocardial
 131 infarction.^{8,9}

132 Why does this occur? Evidence suggests that the high
 133 prevalence of heart disease among diabetic patients is due
 134 to the deleterious effects of hyperglycemia on cardiovascular
 135 pathophysiology. Studies show that the association between
 136 CVD and blood glucose level is linear, there being no
 137 threshold level that emerges as “high-risk.”^{10–12} Blood
 138 glucose levels below the diabetic and impaired glucose toler-
 139 ance ranges continue to have negative effects on cardiovas-
 140 cular outcomes.^{10–12} This may be due to the role played by
 141 chronic hyperglycemia on the development of atheroscle-
 142 rosis through cellular and molecular abnormalities in
 143 multiple relevant cell types.⁷ Pathogenic mechanisms linking
 144 high blood glucose levels with atherosclerosis include endo-
 145 thelial cell dysfunction, increased generation of glycosylated
 146 products, oxidative stress, and impaired vasodilation. Collec-
 147 tively, these abnormalities increase the occurrence and
 148 severity of thrombosis, vessel narrowing, and occlusion.^{7,12}
 149 Therefore, in addition to the microvascular dysfunction
 150 caused by diabetes (retinopathy, nephropathy, and neurop-
 151 athy),² hyperglycemia accelerates macrovascular patholo-
 152 gies such as atherosclerosis and contributes to sequelae
 153 such as myocardial infarction, stroke, peripheral vascular
 154 disease, and heart failure.^{6,7,13}

155 Diabetes often coexists with other well-established risk
 156 factors for CVD such as hypertension and dyslipidemia. Indi-
 157 vidualy, hypertension is the most common cardiovascular
 158 risk factor¹⁴ and a modifiable risk factor with great impact
 159 on CVD burden and mortality.¹⁵ Fortunately, several studies
 160 have demonstrated that pharmacological control of blood

161 pressure (BP) leads to reductions in harmful cardiovascular
 162 outcomes such as stroke and myocardial infarction, espe-
 163 cially in circumstances of secondary prevention.^{16,17} Simi-
 164 larly, dyslipidemia is another key risk factor for CVD in
 165 which the management of blood lipid levels is effective in
 166 improving cardiovascular outcomes,^{18,19} particularly in
 167 survivors of an acute coronary event.²⁰ However, in stark
 168 contrast to the indisputable cardiovascular benefits of treat-
 169 ing hypertension and dyslipidemia, the benefits of lowering
 170 blood glucose to reduce macrovascular disease have been
 171 difficult to demonstrate.²¹

172 173 **Cardiovascular Effects of Drugs Commonly Used** 174 **for the Treatment of Diabetes** 175

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

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

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

205 206 *Sulfonylureas and Potassium Channels* 207

208 Sulfonylureas have been prescribed for the treatment of
 209 type 2 diabetes for more than 50 years and include agents
 210 such as glibenclamide and glimepiride.³⁷ Sulfonylureas act
 211 on pancreatic islet β -cells to stimulate insulin release by
 212 binding to the β -cell sulfonylurea receptor and blocking
 213 ATP-dependent potassium channels. This in turn leads to³⁸
 214 a calcium ion influx and activation of calcium-dependent
 215 proteins that regulate insulin secretion (see also glucagon-

216 **Table**217 **Cardiovascular effects of anti-diabetic drugs**

218 Drug	219 Protective effect		220 Mechanism of action	221 References
	222 Non-diabetic	223 Diabetic		
224 Insulin	225 +	226 +	227 Survival kinases; opens mitochondrial K _{ATP} channel; pharmacological preconditioning	228 Libby et al ³¹ 229 Sack et al ³⁵
230 Sulfonylureas	231 –	232 –	233 Closes mitochondrial K _{ATP} channels; inhibits ischemic preconditioning	234 Brady et al ²³ 235 Garratt et al ²⁷
236 Biguanides (metformin)	237 ±	238 ±	239 <u>Activates AMPK</u> ; increases glucose uptake; induces platelet thrombosis and lactic acidosis	240 Davis et al ²⁶ Q12 241 Olsson et al ³³ 242 Calvert et al ²⁴
243 α-Glycosidase inhibitors	244 ?	245 –	246 Impairs glucose homeostasis in ischemic hearts	247 Liao et al ³⁰
248 Rosiglitazone	249 +	250 ±	251 PPARγ agonists have anti-inflammatory effects; increase glucose uptake; increase fluid retention;	252 Lautamaki et al ²⁹ 253 Home et al ²⁸
254 Pioglitazonew	255 ±	256 ±	257 Antioxidants; close mitochondrial-K _{ATP} channel; proarrhythmic	258 Couzin ²⁵ 259 Quast et al ³⁴
260 GLP-1 analogues	261 +	262 +	263 Promotes ischemic preconditioning; increases glucose uptake; NO-dependent vasorelaxation	264 Nikolaidis et al ³² 265 Sokos et al ³⁶ 266 Ban et al ¹⁰⁰
267 DPP4 inhibitors	268 ?	269 ?	270 Unknown	271
272 Amylin analogues	273 ?	274 ?	275 Unknown	276

277 DPP4, dipeptidyl peptidase-4; GLP-1, glucagon-like peptide-1; MAPK, **NO, ?**; PPARγ, peroxisome proliferator-activated receptor. **Q13**

278 like peptide-1 [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 diabetes information system and the Medicines Monitoring Unit 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 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

279 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

277 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

278 Of interest, recently published findings from the Action in Diabetes and Vascular Disease: Preterax and Diamicon Modified Release Controlled Evaluation and United Kingdom Prospective Diabetes Study 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.

279 *Biguanides Are not Bygones*

280 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 United Kingdom Prospective Diabetes Study 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 (RECORD Study) has revealed that in combination with⁶² 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 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 Action to Control Cardiovascular Risk in Diabetes 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

436 6.25-year follow-up, no significant difference in cardiovas-
 437 cular outcomes was seen in the intensive control group
 438 compared with conventional treatment.^{70,72} The available
 439 agents used to treat diabetes have not been conclusively
 440 shown to reduce macrovascular disease, and, in some
 441 instances, their chronic use may promote negative cardio-
 442 vascular outcomes in diabetic subjects, despite improve-
 443 ment in hyperglycemia. Importantly, these adverse
 444 cardiovascular side effects appear in several instances to
 445 be directly due to the mode of drug action.⁵⁶ Together,
 446 these concepts underscore the need for new drugs in dia-
 447 betes that not only address blood glucose levels, but have
 448 salutary effects on the pathophysiology of CVD.

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

457

458

459 **Might Incretin-Targeted Therapeutics Hold** 460 **Promise?**

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

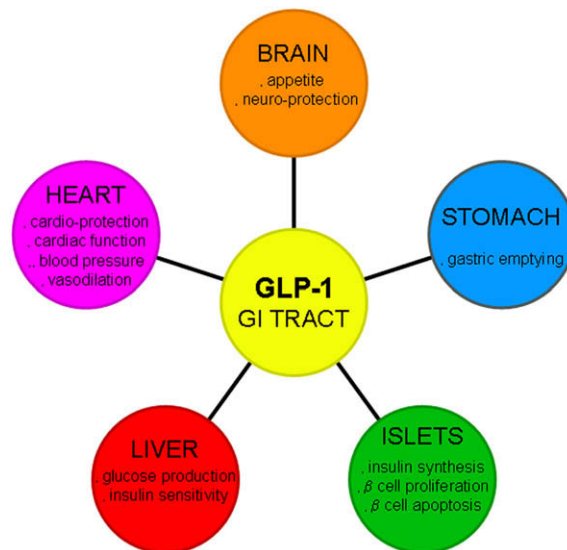
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478 *GLP-1 is an Incretin*

479

480 Although several gut hormones can demonstrate incretin-
 481 like effects, evidence from previous studies in humans and
 482 animal models have suggested that GLP-1 and gastric
 483 inhibitory polypeptide account for almost the entire incretin
 484 effect that facilitates disposal of ingested nutrients. GLP-1
 485 also inhibits glucagon secretion^{75,76} and gastric emptying.⁷⁷
 486 Acute injection of GLP-1 produces a transient reduction in
 487 food intake,⁷⁸ whereas prolonged administration of GLP-1
 488 is associated with weight loss.^{79–82} GLP-1 analogues and
 489 GLP-1 receptor (GLP-1R) agonists also produce modest
 490 weight loss in diabetic subjects (Figure 1).^{83–85}



459 **Figure 1.** Physiological actions of incretins on various tissues.

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

526 *GLP-1 Synthesis, Secretion, and Degradation*

527
 528
 529
 530 GLP-1 is derived from a proglucagon precursor that
 531 encodes GLP-1 and the related proglucagon-derived
 532 peptides: glucagon, GLP-2, oxyntomodulin, and glicen-
 533 tin.⁹⁷ Two forms of GLP-1 are secreted after meal inges-
 534 tion, which differ by a single amino acid: GLP-1(7-37)
 535 and GLP-1(7-36) amide. Both are equipotent and exhibit
 536 identical plasma half-life and biological activities through
 537 the same receptor. However, the majority ($\sim 80\%$) of circu-
 538 lating active GLP-1 appears to be GLP-1(7-36) amide (the
 539 latter being referred to more commonly as GLP-1). GLP-1
 540 is synthesized within the L cells of the intestine. Plasma
 541 levels of GLP-1 are low in the fasting state and rise rapidly
 542 within minutes of food ingestion. GLP-1 secretion from the
 543 distal gut is controlled by neural and endocrine signals initi-
 544 ated by nutrient entry in the proximal gut and by direct
 545 nutrient contact with L cells.^{98,99} After secretion, GLP-1

546 is rapidly cleaved to GLP-1(9-36) at the position 2 alanine
547 by the widely expressed enzyme dipeptidyl peptidase-4
548 (DPP-4). The half-life of exogenously infused bioactive
549 GLP-1 is less than 3 minutes because of enzymatic inacti-
550 vation and renal clearance.

551

552 *The GLP-1 Receptor*

553

554 The actions of GLP-1 are transduced by a 7 transmem-
555 brane-spanning G protein-coupled receptor, the GLP-1R,
556 which is expressed in islet β cells, as well as in the heart
557 and vasculature.¹⁰⁰ Ligand activation of the GLP-1R stim-
558 ulates adenylate cyclase via *G α s*, which leads to an increase
559 in intracellular cyclic adenosine monophosphate (cAMP)
560 and activation of protein kinase A. Hence, GLP-1 acts
561 directly through the cAMP-protein kinase A pathway to
562 enhance and sensitize β cells to glucose-stimulated insulin
563 secretion. Glucose metabolism in the β cell causes an
564 increase in the concentration of ATP and raises the
565 cytoplasmic ATP to ADP ratio, which then leads to depolar-
566 ization of the plasma membrane following closure of
567 ATP-sensitive K^+ channels. This permits opening of
568 voltage-dependent L-type Ca^{2+} channels and increases
569 cytosolic Ca^{2+} , which triggers fusion of insulin-containing
570 secretory vesicles to the plasma membrane. Exocytosis of
571 insulin follows rapidly.

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

584

585 *Drugs Targeting the GLP-1-GLP-1R Axis*

586

587 The rapid degradation and clearance of endogenous and
588 exogenous GLP-1¹⁰⁷ by DPP-4 has spurred the develop-
589 ment of degradation-resistant GLP-1 analogues capable of
590 extended in vivo activity and DPP-4 inhibitors capable of
591 prolonging the activity of endogenous GLP-1. Two
592 different types of GLP-1-targeted drugs, namely a GLP-
593 1R agonist (*Exendin-4*) and a DPP-4 inhibitor (*Sitagliptin*),
594 have been approved in many countries for the treatment of
595 type 2 diabetes, including the United States. A second DPP-
596 4 inhibitor, *vildagliptin*, is also used in many countries.
597 Additional GLP-1 analogues and DPP-4 inhibitors are
598 undergoing clinical trials or are being reviewed for

599 applications as novel drugs. Collectively, if these agents
600 are found to have beneficial effects on the pathophysiology
601 of cardiac and vascular disease, they may emerge with
602 a meaningful advantage over the more traditional diabetes
603 drugs discussed previously.

604

605 *Exendin-4*

606

607 Exendin-4 (Exenatide) is a 39 amino acid naturally
608 occurring GLP-1R agonist isolated from the venom of the
609 lizard *Heloderma suspectum*.¹⁰⁸ The Food and Drug
610 Administration (FDA) approved synthetic Exendin-4 for
611 treatment of type 2 diabetes in April 2005. Exendin-4 binds
612 to and activates the GLP-1R, but is highly resistant to the
613 proteolytic action of DPP-4 in vivo. Therefore, Exendin-4
614 is able to effectively enhance insulin secretion in diabetic
615 subjects,¹⁰⁹ and daily subcutaneous injections are able to
616 significantly reduce blood glucose and glycosylated hemo-
617 globin,¹¹⁰ an accepted measure of blood glucose levels over
618 time. Reported side effects of Exenatide include nausea,
619 vomiting, diarrhea, and loss of appetite and weight. Import-
620 antly, a few cases of acute pancreatitis have also been re-
621 ported in patients treated with Exenatide, prompting new
622 prescribing instructions.

623 *DPP-4 Inhibitors*

624

625 Sitagliptin is an orally active DPP-4 inhibitor that prevents
626 degradation of endogenously released GLP-1, increasing its
627 circulating levels and prolonging its half-life. This agent has
628 been approved for the treatment of type 2 diabetes in many
629 different countries, including the U.S. since 2006. Clinical
630 studies have shown that DPP-4 inhibitors reduce fasting
631 and post-prandial blood glucose levels and glycosylated
632 hemoglobin.^{101,112} The most common known side effects
633 of Sitagliptin include symptoms of an upper respiratory
634 infection, such as stuffy or runny nose, sore throat, and
635 headache.

636 **The Cardioprotective and Vasoactive Actions of 637 GLP-1**

638

639 Studies of cardiovascular pathophysiology have
640 primarily focused on elucidating the cardiac effects of
641 GLP-1 in animal models of heart failure, and two pilot
642 studies have also been conducted in humans. Among the
643 first, conscious dogs with pacing-induced dilated cardiomy-
644 opathy received a 48-hour infusion of recombinant GLP-1
645 (1.5 pmol/kg/min) or saline (3 mL/d). Compared with
646 saline-treated controls, GLP-1 infusions improved left
647 ventricular contractility (92%), stroke volume (102%),
648 and cardiac output (57%).¹¹³ GLP-1-treated dogs exhibited
649 increased myocardial glucose uptake (46%) and oxygen
650 consumption (9.4%), suggesting enhanced oxidative phos-
651 phosphorylation. As such, these authors posited that the
652
653

654 beneficial effects of GLP-1R stimulation are due primarily
 655 to modulation of myocardial metabolism.¹¹³ In a small pilot
 656 study of 10 patients undergoing angioplasty for acute
 657 myocardial infarction, continuous 72-hour infusions of re-
 658 combinant human GLP-1 (1.5 pmol/kg/min) significantly
 659 improved left ventricular ejection fraction (11%) and wall
 660 motion scores (21%) compared with untreated controls.³²
 661 Interestingly, these improvements in cardiovascular func-
 662 tion were sustained, remaining detectable in some patients
 even months after cessation of GLP-1 administration. The
 663 benefits of GLP-1 were independent of infarct location or
 664 history of diabetes; however, the molecular and cellular
 665 mechanisms underlying the observation were not
 666 explored.³² Similarly, a 5-week course of GLP-1 infusion
 667 improved left ventricular ejection fraction (4.4% to 7.7%;
 668 diabetics/nondiabetics and exercise capacity (VO₂-max:
 669 3.2 to 3.3 mL/kg/min) in a pilot study of both diabetic
 670 and nondiabetic subjects with congestive heart failure.³⁶
 671 Finally, preoperative treatment with GLP-1 resulted in gly-
 672 cemic control and comparable hemodynamic recovery
 without high-dose insulin or inotropes following coronary
 artery bypass.¹¹⁴

673 Bose et al demonstrated that treatment with GLP-1
 674 (4.8 pmol/kg/min in vivo; 0.3 nmol in vitro) significantly
 675 reduced infarct size¹¹⁵ and that this protective effect was
 676 blocked by the GLP-1R antagonist exendin(9-39), and by
 677 inhibitors of cAMP, phosphoinositide 3-kinases, and p42/
 678 44 MAPK in both isolated perfused rat hearts and in
 679 a whole animal model of ischemia-reperfusion myocar-
 680 dial injury.¹¹⁵ Intriguingly, these data implicate *multiple*
 681 downstream signaling pathways, each of which appears
 682 critical for mediating the cardioprotective effects of
 683 GLP-1. Most importantly, the benefits of GLP-1 in
 684 perfused hearts (ie, in vitro) appear to rule out an essen-
 685 tial role for indirect (ie, noncardiac) effects of GLP-1 on
 686 myocardial protection.

688 **Cardioprotective and Vasodilatory Actions of** 689 **GLP-1 are Partly Mediated by Glp1r-Independent** 690 **Pathways**

692 Given the accumulating evidence supporting a cardiopro-
 693 tective role for GLP-1, we sought to explore the precise
 694 mechanisms underlying these beneficial effects. Our origi-
 695 nal hypothesis was that the cardioprotection endowed by
 696 GLP-1 treatment would be derived from activation of the
 697 classic GLP-1R and its putative downstream signaling path-
 698 ways. Hence, we expected that the protective effects of
 699 GLP-1 would be completely absent in mice lacking a func-
 700 tional GLP-1R (Glp1r^{-/-}). Having previously generated this
 701 knockout animal¹¹⁶ and used it to examine the biological
 702 actions of GLP-1 in the pancreatic islet^{117–119} and brain,¹²⁰
 703 we next examined the importance of the GLP-1R in the
 704 cardiovascular system.¹²¹ Our initial report on the cardiac
 705 phenotype of Glp1r^{-/-} 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

697 *More Lessons from Glp1r Knockout Mice*

698 Employing an ex vivo perfused heart preparation and an
 699 ischemia-reperfusion (I/R) injury protocol, we showed that
 700 pretreatment with GLP-1 (0.3 nM) significantly enhanced
 701 the recovery of cardiac function (as determined by left
 702 ventricular developed pressure), and decreased cardiomyo-
 703 cyte necrosis (as measured by LDH release) after I/R.^{100, 106}
 704 Interestingly, pretreatment with the GLP-1R agonist Exen-
 705 din-4 (5 nM) resulted in a similar degree of cardioprotec-
 706 tion from I/R injury in hearts of wild-type mice, but
 707 required a 10-fold higher dose as compared with native
 708 GLP-1. We were particularly surprised to discover that
 709 the protective effects of native GLP-1 were preserved in
 710 mice lacking a functional GLP-1R. Together with data
 711 showing that the salutary effects of Exendin-4 (5 nM)
 712 were significantly reduced but not absent in Glp1r^{-/-} hearts,
 713 this finding strongly suggested the existence of a GLP-1R-
 714 independent signaling pathway for cardioprotection.

715 *An Alternate GLP-1 Receptor?*

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

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

729 We next turned our attention to examining the potential
 730 role of GLP-1(9-36), the DPP-4-generated metabolite of
 731 GLP-1, as a potentially critical intermediary in GLP-1-
 732 mediated and GLP-1R-independent protection against I/R
 733 injury. Although GLP-1(9-36) has traditionally been
 734 considered as either an inactive or weak agonist of the
 735 GLP-1R, the vast majority of previous studies examining
 736 the cardiovascular effects of GLP-1 have been performed
 737 without inhibition of the highly active DPP-4 enzyme,
 738 making it difficult to determine whether the cardiovascular
 739 effects of GLP-1 were derived from GLP-1 itself, GLP-1(9-
 740 36), or both. Two recent reports ascribe protective cardio-
 741 vascular 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.^{125,2} Second, Sonne et al
 demonstrated that administration of GLP-1(9-36) following
 global ischemia significantly improved left ventricular pres-
 sure, 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 *Glp1r*^{-/-}
 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

779

GLP-1 Exhibit Vasodilatory Action

781

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
Glp1r^{-/-} mice, suggesting a *Glp1r*-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 monophos-
 phate (cGMP) release. Also, these vasodilatory actions
 were attenuated by preincubation of vessels with NG-nitro-
 L-arginine, a nonselective NOS inhibitor, suggesting that at
 least part of their vasodilatory mechanism is NO/cGMP-
 dependent. Unlike GLP-1 or GLP-1(9-36), Exendin-4 did
 not produce any vasodilatation 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 phos-
 phodiesterase-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 postis-
 chemic cardiac function was improved when atrial natri-
 uretic 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 sup-
 ported that the cardiovascular effects of GLP-1 are partly
 mediated by a GLP-1(9-36)-dependent pathway. Import-
 antly, significant degrees of vasodilation and cardioprotec-
 tion 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 treat-
 ment.^{137–140} Most recently, Green et al demonstrated that
 five structurally related peptides 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
 GLP-1(9-36)-dependent effects via a putative alternate

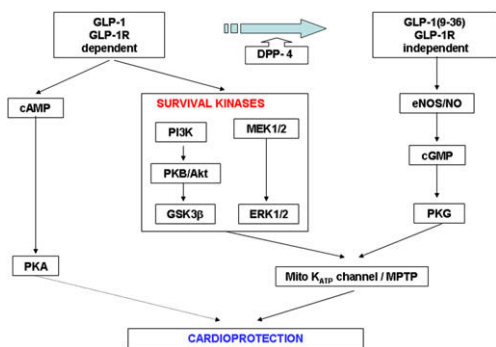


Figure 2. Putative cardioprotective mechanisms of GLP-1 receptor agonists. cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; DPP-4, dipeptidyl peptidase 4; eNOS, endothelial nitric oxide synthase; ERK1/2, extracellular signal-regulated kinases; GLP-1R, glucagon like peptide-1 receptor; GSK3 β , glycogen synthase kinase 3 beta; MEK1/2, mitogen-activated protein kinase kinase; Mito KATP channel, mitochondrial adenosine triphosphate-dependent potassium channel. MPTP, mitochondrial permeability transition pores. NO, nitric oxide; PI3 K, phosphoinositide 3-kinases; PKA, protein kinase A; PKB/Akt, protein kinase B; PKG, protein kinase G.

receptor of ischemic “postconditioning,” and vasodilation 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.

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111,125.

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