Cardiovascular Actions and Clinical Outcomes With Glucagon-Like Peptide-1 Receptor Agonists and Dipeptidyl Peptidase-4 Inhibitors

ABSTRACT: Potentiation of glucagon-like peptide-1 (GLP-1) action through selective GLP-1 receptor (GLP-1R) agonism or by prevention of enzymatic degradation by inhibition of dipeptidyl peptidase-4 (DPP-4) promotes glycemic reduction for the treatment of type 2 diabetes mellitus by glucose-dependent control of insulin and glucagon secretion. GLP-1R agonists also decelerate gastric emptying, reduce body weight by reduction of food intake and lower circulating lipoproteins, inflammation, and systolic blood pressure. Preclinical studies demonstrate that both GLP-1R agonists and DPP-4 inhibitors exhibit cardioprotective actions in animal models of myocardial ischemia and ventricular dysfunction through incompletely characterized mechanisms. The results of cardiovascular outcome trials in human subjects with type 2 diabetes mellitus and increased cardiovascular risk have demonstrated a cardiovascular benefit (significant reduction in time to first major adverse cardiovascular event) with the GLP-1R agonists liraglutide (LEADER trial [Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Ourcome Results], −13%) and semaglutide (SUSTAIN-6 trial [Trial to Evaluate Cardiovascular and Other Long-term Outcomes with Semaglutide], −24%). In contrast, cardiovascular outcome trials examining the safety of the shorter-acting GLP-1R agonist lixisenatide (ELIXA trial [Evaluation of Lixisenatide in Acute Coronary Syndrom]) and the DPP-4 inhibitors saxagliptin (SAVOR-TIMI 53 trial [Saxagliptin Assessment of Vascular Outcomes Recorded in Patients With Diabetes Mellitus-Thrombolysis in Myocardial Infarction 53]), alogliptin (EXAMINE trial [Examination of Cardiovascular Outcomes With Alogliptin Versus Standard of Care in Patients With Type 2 Diabetes Mellitus and Acute Coronary Syndrome]), and sitagliptin (TECOS [Trial Evaluating Cardiovascular Outcomes With Sitagliptin]) found that these agents neither increased nor decreased cardiovascular events. Here we review the cardiovascular actions of GLP-1R agonists and DPP-4 inhibitors, with a focus on the translation of mechanisms derived from preclinical studies to complementary findings in clinical studies. We highlight areas of uncertainty requiring more careful scrutiny in ongoing basic science and clinical studies. As newer more potent GLP-1R agonists and coagonists are being developed for the treatment of type 2 diabetes mellitus, obesity, and nonalcoholic steatohepatitis, the delineation of the potential mechanisms that underlie the cardiovascular benefit and safety of these agents have immediate relevance for the prevention and treatment of cardiovascular disease.

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Glucagon-like peptide-1 (GLP-1) was initially discovered as an insulinotropic hormone produced in and secreted from the gut after food intake.[1] It has received attention because of its role in the physiology of glucose metabolism (ie, its function as an incretin[2]) but more so as a parent compound mediating the actions of 2 classes of glucose-lowering medications used in the treatment of type 2 diabetes mellitus (T2D), GLP-1 receptor (GLP-1R) agonists, and dipeptidyl peptidase-4 inhibitors (DPP-4Is).[1] GLP-1R agonists, either small peptides or much larger peptidomimetics, mediate their glucoregulatory actions by a single GLP-1R. In contrast, inhibitors of the protease DPP-4 prevent the degradation and inactivation of both GLP-1 and the incretin hormone glucose-dependent insulinotropic polypeptide.[1]

GLP-1R agonists and DPP-4Is are approved for the treatment of hyperglycemia in patients with T2D.[2] Although glycemic control reduces the microvascular complications of diabetes mellitus (neuropathy, nephropathy, and retinopathy), the relationship between glucose control and reduction of macrovascular events is more challenging.[3] It is notable that incretin-based therapies (GLP-1R agonists [GLP-1RAs] and DPP-4Is) exert multiple nonglycemic actions in the cardiovascular system, heightening the interest in their potential for cardiovascular benefit.[4–6] The recent findings that 2 GLP-1RAs, liraglutide[7] and semaglutide,[8] significantly reduced the combined primary outcome of 3 point major adverse cardiovascular events in large cardiovascular outcome trials elevates the importance of understanding how activation of the GLP-1R translates into clinical cardiovascular benefit. The purpose of the present review is to summarize the literature on indirect (through lowering glucose and modifying known cardiovascular risk factors) and direct (through stimulating GLP-1Rs and inhibition of DPP-4) effects of (1) GLP-1, (2) GLP-1RAs, and (3) DPP-4Is on the heart and blood vessels. Herein we discuss concepts of incretin action in the context of results of cardiovascular outcomes trials with DPP-4Is and GLP-1RAs and, wherever possible, link underlying mechanisms to observed clinical benefits.

GLP-1RS IN THE CARDIOVASCULAR SYSTEM AND EFFECTS ELICITED BY STIMULATING WITH GLP-1, GLP-1RAS, OR DPP-4IS (PRECLINICAL STUDIES)

GLP-1Rs in the Cardiovascular System

GLP-1R expression has been detected in various cardiovascular tissues and cell types at the mRNA and protein levels. Although native GLP-1 improves endothelial function, augments ventricular contractility, enhances myocardial glucose uptake, and exerts cytoprotective and metabolic actions on blood vessels and cardiomyocytes, the endogenous canonical GLP-1R is not highly expressed in many of the cell types responsive to GLP-1 or GLP-1RAs. Hence, some of the well-described actions of GLP-1 in preclinical studies may reflect indirect mechanisms or the actions of ≥1 GLP-1 degradation products acting through GLP-1R-independent mechanisms. Details are summarized in Table I in the online-only Data Supplement, which highlights well-documented effects on the heart (contractile function, substrate supply, coronary and myocardial blood flow, rate control), blood pressure, and platelet aggregation. Because the present review is focused on human studies, we refer to Table I and accompanying text in the online-only Data Supplement and several recent reviews for details of preclinical studies.[4–6,9,10]

Potential Mechanisms Explaining Biological Effects of N-Terminal GLP-1 Fragments GLP-1 [9–36] Amide, GLP-1 [9–37], or GLP-1 [28–36] Amide

Considerable evidence supports biological activity for N-terminally truncated GLP-1 peptides, principally GLP-1 [9–36] amide[10,11] and GLP-1 [28–36] amide[12] in the cardiovascular system. Although a distinct receptor for these peptides has not been identified, they successfully target cytoplasmic and mitochondria-linked pathways, leading to a reduction of reactive oxygen species in hepatocytes, endothelial cells, and cardiomyocytes.[10,11] Moreover, GLP-1 [28–36] directly activates prosurvival kinases in the ischemic mouse heart or vascular cells through mechanisms linked to soluble adenylate cyclase and cAMP generation in isolated cardiomyocytes ex vivo.[12] Hence, studies using native GLP-1 may be associated with activation of dual cardiovascular pathways mediated through the classical GLP-1R and nonclassical cAMP-mediated pathways activated by truncated peptides converging on cardiomyocyte and vascular protection.[10]

DPP-4 in the Cardiovascular System

DPP-4 is widely expressed in most cells and tissues and exhibits enzymatic activity against dozens of chemokines and peptide hormones with roles in inflammation, vascular function, stem cell homing, and cell survival.[13] DPP-4 exhibits exopeptidase activity through its 2 principal molecular forms, a membrane-tethered 766 amino acid protein with a small intracellular tail and a soluble form that is 39 amino acids smaller, devoid of the short membrane spanning domain and intracellular tail, and yet otherwise structurally identical.[13] Although soluble DPP-4 exerts vascular, immune, and proinflammatory actions independent of its catalytic activity, the

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majority of the experimental literature has studied the importance of DPP-4-mediated peptide cleavage in the pathophysiology and treatment of cardiovascular disease.

Attribution of mechanism(s) linking reduction of DPP-4 activity to attenuation of cardiovascular injury or preservation of cardiovascular function is difficult for several reasons. First, DPP-4 cleaves dozens of substrates simultaneously, initiating complex changes in multiple signaling pathways.[4,5] Second, the majority of DPP-4 substrates circulate at low levels and are difficult to quantitate. Third, highly sensitive and specific assays distinguishing full length from DPP-4-cleaved peptides are generally not commercially available. Hence, measurements of total immunoreactive peptide detect a mixture of cleaved versus intact substrates. Fourth, many of the DPP-4-cleaved peptide metabolites retain biological activity in the cardiovascular system, albeit through different receptors and signaling pathways. Hence, DPP-4 simultaneously inactivates and potentiates the activity of numerous cardioactive substrates.[5,13] Last, only a few highly selective antagonists for DPP-4 peptide substrates are available, and these reagents have not been widely used in cardiovascular studies.

Preclinical Effects in Myocardial Infarction Models and Cardiovascular Function

When myocardial infarction is experimentally induced by occluding (ligating) a coronary artery, the myocardial area receiving blood supply through the vessel to be occluded can be defined as an area at risk, and the resulting area of necrosis can be identified by specific staining methods.[14] Administration of GLP-1, GLP-1RAs (eg, exenatide, liraglutide), and DPP-4Is (eg, sitagliptin, vildagliptin, alogliptin) reduces the resulting necrosis (relative to the area at risk), as summarized in Figure I in the online-only Data Supplement. Examples encompass in vivo and ex vivo (isolated perfused heart) studies, studies in rodents and larger mammals, and with various pharmacological agents (GLP-1 [7–36 amide], DPP-4Is, and GLP-1RAs) (Figure I in the online-only Data Supplement). Additional studies examining effects of the GLP-1RAs exenatide,[15] lixisenatide,[16] and albiglutide[17] and the DPP-4Is sitagliptin[18] and linagliptin[19] have been published. Although occasional reports do not replicate these findings (eg, with liraglutide in a porcine model[20]), the majority of studies found a significant reduction in the necrotic area in hearts of animals treated with GLP-1 or GLP-1RAs (Figure I in the online-only Data Supplement). The cardioprotective effects of GLP-1 can be inhibited by the specific GLP-1RA exendin [9–39]. Thus, these effects seem to be mediated by an interaction with the canonical GLP-1R.[14] More details are described in the online-only Data Supplement.

CARDIOVASCULAR ACTIONS IN HUMANS

Table 1 summarizes human studies examining cardiovascular function or changes in renal function, lipoprotein metabolism, and hepatic fat accumulation.

GLP-1R in Human Cardiovascular Tissues

The atrial expression of the GLP-1R protein was identified in nonhuman primate and human hearts using a highly specific monoclonal antibody, localizing an immunoreactive GLP-1R protein to cells within the sinoatrial node.[21] Nevertheless, some studies have detected partial *GLP-1R* mRNA transcripts by reverse transcription polymerase chain reaction techniques using RNA isolated from human ventricles, although GLP-1RAs such as exenatide failed to augment contractility in the majority of isolated strips from human ventricles in the same experiments.[27] RNASeq analyses have detected the presence of *GLP-1R* mRNA transcripts in RNA from human left ventricles (http://www.gtexportal.org/home/gene/GLP1R). Hence, these findings imply that under some circumstances, transcriptional or translational control may dictate whether a ventricular *GLP-1R* mRNA transcript is expressed and gives rise to functional GLP-1R protein in the human heart (including the working myocardium in atria and ventricles). The presence or absence of a functional GLP-1R in human coronary arteries is not clearly established.[6]

Cardiac Output

Intravenous GLP-1 at a pharmacological dose improved left ventricular function, maximum oxygen uptake, and physical performance in subjects with congestive heart failure.[25] Likewise, intravenous exenatide (GLP-1RA) improved cardiac index and pulmonary capillary wedge pressure and reduced atrial natriuretic peptide.[28] However, in vitro, exenatide increased contractility in human atrial but not ventricular myocardium.[27] Larger randomized controlled clinical trials with albiglutide or liraglutide failed to demonstrate any beneficial effect of sustained GLP-1RA treatment in human subjects with moderate to severe heart failure and reduced ejection fraction,[29,30] independent of the presence or absence of diabetes mellitus. In patients with advanced heart failure, liraglutide did not improve a composite end point of cardiovascular events that included changes in N-terminal pro-brain natriuretic peptide. A numeric but statistically nonsignificant increase in mortality and hospitalization for heart failure was detected (hazard ratio [HR], 1.30; 95% confidence interval [CI], 0.92–1.83; *P*=0.14), indicating a potential for harm in patients with reduced ejection fraction and a prior history of hospitalization for heart failure. It is possible that this may be related to

Table 1. Effects of Stimulating GLP-1 Receptors With GLP-1, GLP-1 Receptor Agonists, or DPP-4 Inhibitors in Human Studies, Which Lead to a Modified Cardiovascular Function (Directly or Indirectly)

Organ	Effect(s) on	GLP-1 [7–36 Amide] or [7–37]	GLP-1 Receptor Agonists	DPP-4 Inhibitors
Heart	Myocardial glucose uptake	• Intravenous GLP-1 (pharmacological dose): ≈[22]	• Exenatide (intravenous, pharmacological dose, type 2 diabetes mellitus, no CAD): ≈[23]	• Sitagliptin (subjects without diabetes mellitus, subjects with nonischemic cardiomyopathy): ↑[24]
	Left ventricular function	• Intravenous GLP-1 (pharmacological dose, 5 wk): LVEF ↑, VO_2 max. ↑, 6-min walk, distance ↑[25] • Improved LVEF not confirmed at lower dose of GLP-1[26]	• Exenatide: In vitro contractility of atrial, but not ventricular human myocardium ↑[27]; intravenous: cardiac index ↑, PCWP ↑, and ANP ↓[28] • Albiglutide: no significant effects[29] • Liraglutide: trend for reduced rate of hospitalization for congestive heart failure (LEADER)[7]; however, trends for worse outcomes (not significant) in dedicated heart failure trials[30,31]	• Sitagliptin (chronic congestive heart failure): left ventricular diastolic function ↑[32] • Rate of hospitalization for congestive heart failure ≈ (TECOS)[33] • Saxagliptin: rate of hospitalization for congestive heart failure ↑ (significant) SAVOR-TIMI 53[34,35] • Alogliptin: rate of hospitalization for congestive heart failure ↑ (nonsignificant) EXAMINE[36,37] • Vildagliptin: trend to reduced left venticular function (VIVIDD trial, unpublished)
	Cardioprotection against ischemia/ myocardial stunning	• Intravenous GLP-1 (pharmacological dose, dobutamine-induced stress) LVEF ↑, regional contractility ↑[38,39] • Coronary balloon occlusion: preserved left ventricular function[38,40] • 72 h after acute myocardial infarction: LVEF ↑, regional wall motility ↑[41]	• ST-segment elevation myocardial infarction: intravenous exenatide: salvage index (non-necrosed proportion of area at risk) ↑[42] • Subcutaneous exenatide: infarct size ↓[43] • Liraglutide preserved LVEF after PCI[44] • Non–ST-segment elevation myocardial infarction: liraglutide-preserved LVEF after PCI[45]	• Sitagliptin (dobutamine-induced stress): LVEF ↑, regional contractility ↑. Preferential effect in ischemic segments[46,47]
	Heart rate	• Intravenous GLP-1: ↑ (small), no decrease in vagal control[48]	• ↑ by 2–3 beats per min[49,50] • Sympathetic activation with exenatide?[51]	• Not reported in a study demonstrating lowering in systolic blood pressure by ≈ 2 mm Hg[52]
Peripheral arteries	Angiogenesis, endothelial cell proliferation	• New vessel formation from human endothelial cells improved by high doses of GLP-1[53]	• Exenatide-stimulated proliferation of human coronary artery endothelial cells[54]	• Not reported
	Endothelium-derived vasodilation (NO production)	• Endothelial nitric oxide synthase ↑ in HUVECs[55] • Intravenous GLP-1 (pharmacological dose): acetyl choline–induced vasodilation ↑ in healthy subjects[56] and in type 2 diabetes mellitus with stable CAD[57]	• Exenatide: endothelial nitric oxide synthase in HUVECs ↑[58]; postprandial endothelial function ↑[59] • Liraglutide: endoplasmic reticulum stress (induced by hyperglycemia) ↓[60] and TNF^{α}-induced oxidative stress ↓ and inflammation ↓ in HUVECs[61]; eNOS ↑, endothelin-1 expression ↓[62] • Liraglutide: Acetyl choline–mediated forearm blood flow (↑) (n.s.)[63]	• Sitagliptin: reactive hyperemia peripheral artery tonometry index ↑[64], flow- mediated vasodilation (type 2 diabetes mellitus) ↑[65] • Effect of DPP-4 inhibition on endothelial function not confirmed by other studies[66,67]
	Endothelium-independent vasodilation	• Nitroprusside-induced forearm vasodilation not augmented by intravenous GLP-1 (pharmacological dose)[56]	• Not reported	• Nitroglycerin-mediated dilatation not changed by sitagliptin[65]
	Anti-atherosclerotic effects	• No immediate effects	• Liraglutide: intimamedia thickness ↓ over 8 months[68]	• Sitagliptin,[69] linagliptin[70]: intimamedia thickness progression ↓
Blood pressure	Systolic	• Transient increase with GLP-1 (intravenous; pharmacological dose: transient ↑[71]; physiological dose: ≈[72])	• ↓ by 2–3 mmHg[49,50]	• Occasional reports of lowering systolic blood pressure in hypertensive subjects[52]
	Natriuretic peptides	• ANP ≈ (n.s.)[71,73]	• Liaglutide: pro-ANP ↓,[74] but ANP and pro-BNP ≈[75,76]; ANP ↑ and BNP ↑ also reported[77]	• Not reported

(*Continued*)

Table 1. Continued

Organ	Effect(s) on	GLP-1 [7–36 Amide] or [7–37]	GLP-1 Receptor Agonists	DPP-4 Inhibitors
Renal function	Glomerular filtration	• Acutely ↑[78]	• Exenatide. ≈[79] • Lixisenatide: ≈[80] • Liraglutide: ≈[7]	• Generally no significant effect[36] • Sitagliptin: minor ↓ in the TECOS trial[33]
	Albumin excretion	• No immediate effects known	• Liraglutide: ↓[7,74] • Lixisenatide: ↓ (P=0.004)[80]	• Saxagliptin ↓,[34] linagliptin ↓[81]
Metabolic milieu	Hyperglycemia	• Plasma glucose ↓[82]	• See Figure 2	• See Figure 2
	Fasting lipoproteins/lipid concentrations	• No immediate effect, nonesterified fatty acids ↓ (transient)[82]	• See Figure 2	• See Figure 2
	Postprandial lipid concentrations	• Postprandial triglycerides ↓ (deceleration of gastric emptying)[83]	• Exenatide,[84] liraglutide[85]: triglycerides ↓, apolipoprotein B-48 ↓,[85] and in chylomicron remnant lipids ↓[84]	• Sitagliptin,[86] vildagliptin,[87] and alogliptin[88]: triglycerides ↓, apolipoprotein B-48 ↓
Liver	Hepatic fat deposition (hepatic steatosis, NAFLD)	• No effects reported	• Mechanistic study describes the role of exenatide and liraglutide in stimulating lipoautophagy (macroautophagy and chaperone-mediated autophagy) in preventing apoptiosis, fat-induced hepatocyte death, and progression to hepatic fibrosis and cirrhosis[89] • Exenatide: better reversal of fatty liver (ultrasonography) than with insulin[90] • Liraglutide: resolution of definite nonalcoholic steatohepatitis (histology) vs placebo ↑[91]	• Vildagliptin: hepatic triglyceride content ↓ vs placebo[92] • Sitagliptin: ≈ vs placebo[93]
Inflammatory responses	Reactive oxygen species/ oxidative stress	HUVECs: ROS ↓[94]	• Exenatide: ROS generation ↓[95], anti-oxidative potential in human monocytes/ macrophages ↑[96]	• No effects reported
	NF-κB binding/activation	No immediate effects reported	• Exenatide: nuclear factor-κB binding (mononuclear blood cells) ↓[95]	• Sitagliptin: nuclear factor-κB binding (mononuclear blood cells) ↓[97]
	Expression of inflammatory cytokines in mononuclear cells	IL-6 ↓[98]	• Exenatide: TNFα ↓, IL-1ß ↓, etc.[95] • Liraglutide: TNFα ↓, IL-1ß ↓, IL-6 ↓, etc.[99]	• Sitagliptin: significant reduction in IL-6, IL-18, sICAM-1, E-selectin[100]; significant reduction in TNFα, TLR-4, TLR-2, CCR-2[97]
	C-reactive protein	No immediate effects reported	• Exenatide: ↓ by 61%[101] • Liraglutide ↓ by 23%[102]	• Sitagliptin: ↓ by 44%[100]
	Adiponectin	• No immediate effects reported	• Exenatide: ↑ by 12%[101] • Liraglutide: ↑ by 40%[99]	• Increase more substantial with vildaglptin than with sitagliptin[103]
Platelet function	Platelet aggregation	• No immediate effects reported	• Exenatide: platelet aggregation ↓[104]	• Potential for reduced platelet aggregation(?)[105]
Stem cell homing	SDF-1 stabilization	• No immediate effects reported	• No immediate effects reported	• Circulating endothelial progenitor cells (reduced in subjects with type 2 diabetes) enhanced after 4 weeks of treatment with sitagliptin[106] • Benefits of improved stem cell homing not supported by results of the SITAGRAMI study (sitagliptin for 28 days and granulocyte-colony stimulating factor for 5 days after acute myocardial infarction)[107]

Ach indicates acetyl choline; ANP, atrial natriuretuc peptide; BNP, brain-type natriuretic peptide; CAD, coronary artery disease; CCR-2, chemokine receptor type 2; DPP-4, dipeptidyl peptidase-4; eNOS, endothelial nitric oxide synthase; EXAMINE, Examination of Cardiovascular Outcomes with Alogliptin versus Standard of Care; GLP-1, glucagon-like peptide-1; HUVECs, human umbilical vein endothelial cells; IL-1ß, interleukin 1ß; IL-6, interleukin 6; IL-18, interleukin-18; LEADER, Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results; LVEF, left ventricular ejection fraction; NAFLD, nonalcoholic fatty liver disease; NF-κB, nuclear factor κB; n.s., not significant; PCI, percutaneous coronary intervention; PCWP, pulmonary capillary wedge pressure; ROS, reactive oxygen species; SAVOR-TIMI 53, Saxagliptin Assessment of Vascular Outcomes Recorded in Patients with Diabetes Mellitus-Thrombolysis in Myocardial Infarction; SDF-1, stromal-derived factor-1; sICAM, soluble intercellular adhesion molecule; SITAGRAMI, Sitagliptin Plus Granulocyte-colony Stimulating Factor in Acute Myocardial Infarction; TECOS, Trial to Evaluate Cardiovascular Outcomes after Treatment with Sitagliptin; TLR-2, toll-like receptor-2; TLR-4, toll-like receptor-4; TNFα, tumor necrosis factor α; VIVIDD, Vildagliptin in Ventricular Dysfunction Diabetes; VO_2, velocity of opxygen uptake; ↑, improved, enhanced; ↓, reduced, worsened; and ≈, no significant change.

increases in heart rate or development of arrhythmias.[30] Coronary blood flow was not changed by liraglutide treatment (Table 1). In contrast, in a small pilot study of 18 subjects with T2D treated with sitagliptin for 24 weeks, an improvement in diastolic but not systolic ventricular function was shown.[32] Intravenous GLP-1 and exenatide (GLP-1RA) did not affect myocardial glucose uptake[22,23] (Table 1), whereas treatment with the DPP-4I sitagliptin increased myocardial glucose uptake (in subjects without diabetes mellitus with nonischemic cardiomyopathy[24]; Table 1). Overall, GLP-1RA use in human subjects with or without diabetes mellitus does not appear detrimental to cardiac function, except for patients with advanced heart failure. The effects of DPP-4Is in short-term mechanistic studies have to be reconciled with the observation that the DPP-4 I saxagliptin significantly increased the risk for hospitalization for congestive heart failure in the SAVOR TIMI 53 trial[35] (*vide infra* in the section on clinical trial results).

Ischemic Conditioning

Intravenous GLP-1 improved left ventricular ejection fraction and regional contractility during dobutamine-induced stress and preserved left ventricular function during coronary balloon occlusion.[38,39] Perioperative intravenous infusions of GLP-1 during and after aortocoronary bypass grafting did not result in changes in left ventricular ejection fraction or cardiac index but reduced the need for inotropic medications.[108] In a pilot study with a small number of patients with and without diabetes mellitus, 72 hours of intravenous GLP-1 infusion to patients with acute myocardial ischemia undergoing percutaneous revascularization improved left ventricular ejection fraction and regional wall motility.[41] Similar evidence with GLP-1RAs suggests an improved salvage of myocardium at risk for necrosis with intravenous exenatide[42] and a reduced infarct size with subcutaneous exenatide.[43] Liraglutide treatment reduced the resulting necrotic area[109] and improved left ventricular ejection fraction after percutaneous intervention for ST-segment elevation[44] and non-ST-segment elevation myocardial infarction.[45] The DPP-4I sitagliptin improved left ventricular ejection fraction and regional contractility during dobutamine-induced stress,[46] with a preferential effect in ischemic segments of the heart.

Heart Rate

Changes in heart rate have not been consistently described with intravenous infusions of GLP-1, at both physiological and pharmacological concentrations. However, a small rise in heart rate (usually by 2–3 beats per minute) has been described in short-term controlled studies with a GLP-1RA (Figure 1). Studies using ambulatory 24-hour monitoring have found larger average changes in heart rate of up to 9 beats per minute.[110] Also, 24-hour monitoring shows that variation in duration of heart rate changes may exist depending on the exposure to the GLP-1RA. Thus, long-acting GLP-1RAs elevate heart rate for 24 hours, whereas short-acting compounds only lead to a transient rise in heart rate for the period characterized by effective drug levels.[110] The relative contributions of the autonomic nervous system (as suggested by a study of exenatide[51]), versus the direct actions of GLP-1RAs on the GLP-1R located in the sinoatrial node,[21] on heart rate (Figure 1) is difficult to ascertain in human subjects (Table 1). The functional consequences, if any, of longer term increases in heart rate in subjects treated with GLP-1RAs are not entirely known. Although such an increment in heart rate does not seem to prevent overall beneficial results in terms of clinical end points in the LEADER trial (Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results)[7] and SUSTAIN-6 trial (Trial to Evaluate Cardiovascular and Other Long-term Outcomes with Semaglutide in Subjects with Type 2 Diabetes),[8] we do not know whether patients with more marked increments in heart rate exhibit different outcomes compared with those with no or less marked increments. Additional analyses will be required to clarify the clinical consequences of changes in heart rate with GLP-1RA treatment.

Effects on the Endothelium

GLP-1 and the GLP-1RA exenatide stimulate proliferation of human endothelial cells in ex vivo studies (Table 1). This finding suggests a possible effect of GLP-1 on new vessel formation, but similar results have not been reported for DPP-4Is. GLP-1 and the GLP-1RAs exenatide and liraglutide increased nitric oxide synthase activity in human endothelial cells[58,62] (Table 1). Furthermore, intravenous GLP-1 improves endothelial function,[56,57] and the GLP-1RA liraglutide augmented acetyl choline-induced vasodilation.[63] The effect of liraglutide alone on endothelial function, which is relatively resistant to degradation by DPP-4 and thus does not form significant amounts of the DPP-4 metabolite, was not significant[65] (Table 1). This finding could mean that peptides structurally related to GLP-1 [9–36] amide or GLP-1 [9–37] are needed for this effect (*vide supra*). Intravenous exenatide increased myocardial blood flow.[23] Exenatide improved endothelial function after a lipid-rich meal[84], and a single subcutaneous dose of exenatide reduced peripheral vascular resistance. The DPP-4I sitagliptin improved flow-mediated vasodilation in subjects with T2D.[65] However, other studies did not confirm beneficial effects of DPP-4Is on endothelial function[66,67] (Table 1). In contrast to endothelium-dependent vasodilation, endothelium-independent vasodilation (induced by nitroglycerin or nitroprusside) has not been changed with either GLP-1[56] or DPP-4Is.[65] Liraglutide reduced endothelin-1, a peptide able to induce vasoconstriction, in

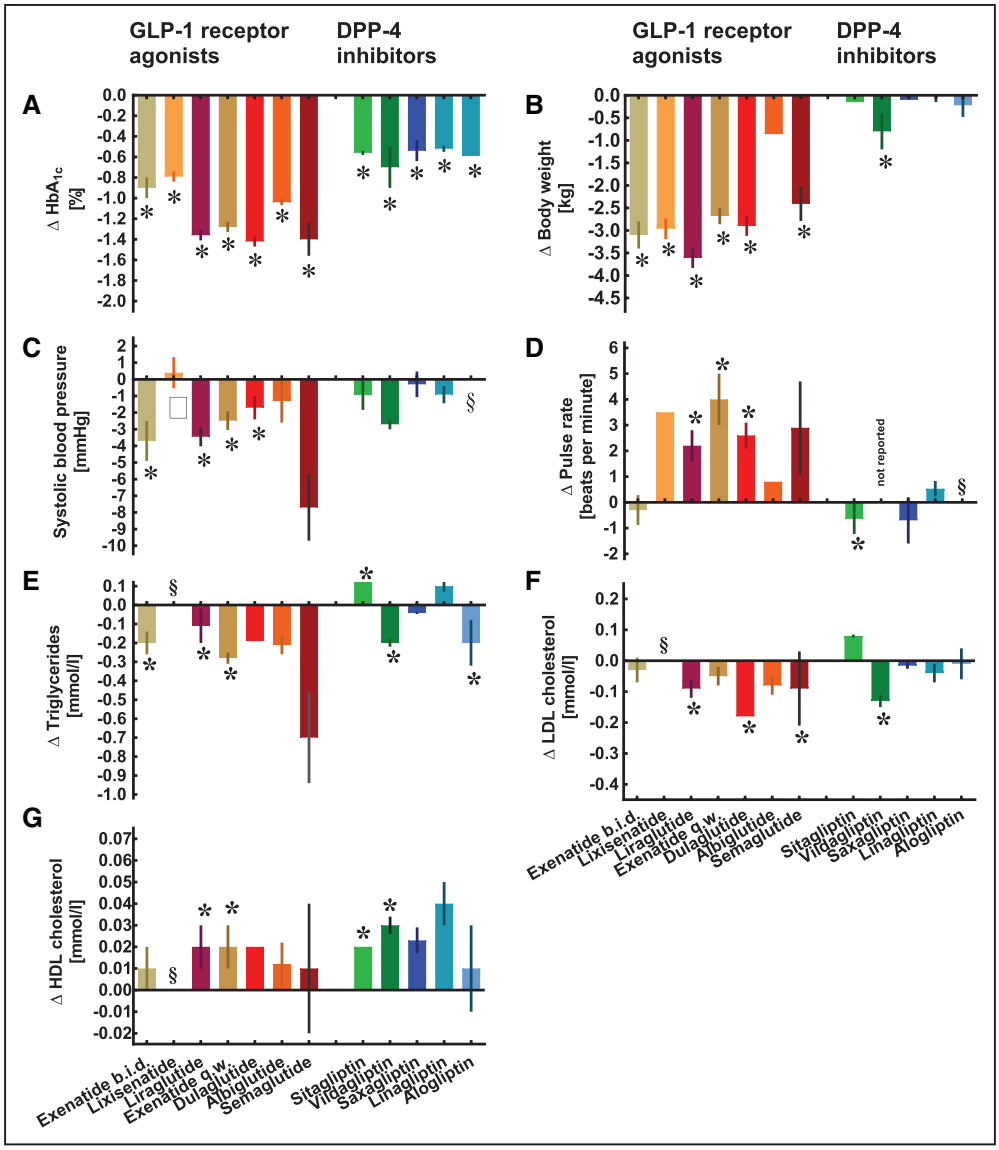

Figure 1. Effects of treatment with glucagon-like peptide-1 (GLP-1) receptor agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors on cardiovascular risk factors as described in placebo-controlled clinical trials using incretin-based medications as monotherapy.

The placebo-subtracted differences to baseline (± standard error of the mean) are shown for glycohemoglobin (**A**), body weight (**B**), systolic blood pressure (**C**), pulse rate (**D**), serum triglycerides (**E**), low-density lipoprotein (LDL) cholesterol (**F**), and high-density lipoprotein (HDL) cholesterol (**G**). BID indicates twice daily; q.w., once weekly. *Significant difference (P<0.05). †Median instead of mean value reported. ‡Based on 24-hour monitoring. §Reported as no relevant change in vital parameters or lipid levels. Data displayed in Figure 2 are from Moretto et al[122] and Simó et al[123] (exenatide BID); Rosenstock et al[124,125] and Meier et al[110] (lixisenatide); Nauck et al,[126] Buse et al,[127] and Dungan et al[128] (liraglutide); Drucker et al[129] and Diamant et al[130] (exenatide q.w.); Nauck et al[131] and Dungan et al[128] (dulaglutide); Pratley et al[132] and Nauck et al[133] (albiglutide); Nauck et al[134] (semaglutide, 0.8 mg/wk, dose initially slowly escalated; semaglutide has not been approved for the treatment of type 2 diabetes; in phase 3, doses of 0.5 and 1.0 mg/wk have been used); Hanefeld et al,[135] Pratley et al[136] and Charbonnel et al[137] (sitagliptin); Dejager et al[138] and Evans et al[139] (vildagliptin); Jadzinsky et al[140] and Rosenstock et al[141] (saxagliptin); Zinman et al[142] (linagliptin); and DeFronzo et al[143] (alogliptin).

human umbilical vein endothelial cells[62] (Table 1). Evidence suggests that with both GLP-1RAs (liraglutide)[68] and DPP-4Is (sitagliptin[69] and linagliptin[70]), treatment may reduce intimamedia thickness or slow the progressive increment in intimamedia thickness, indicating interference with the progression of atherosclerosis. Collectively, although native GLP-1 consistently improves endothelial function independent of changes in insulin or glucose in human studies, insufficient evidence supports a similar benefit on endothelial function in human subjects with diabetes mellitus treated with degradation-resistant GLP-1RAs or DPP-4Is.[10]

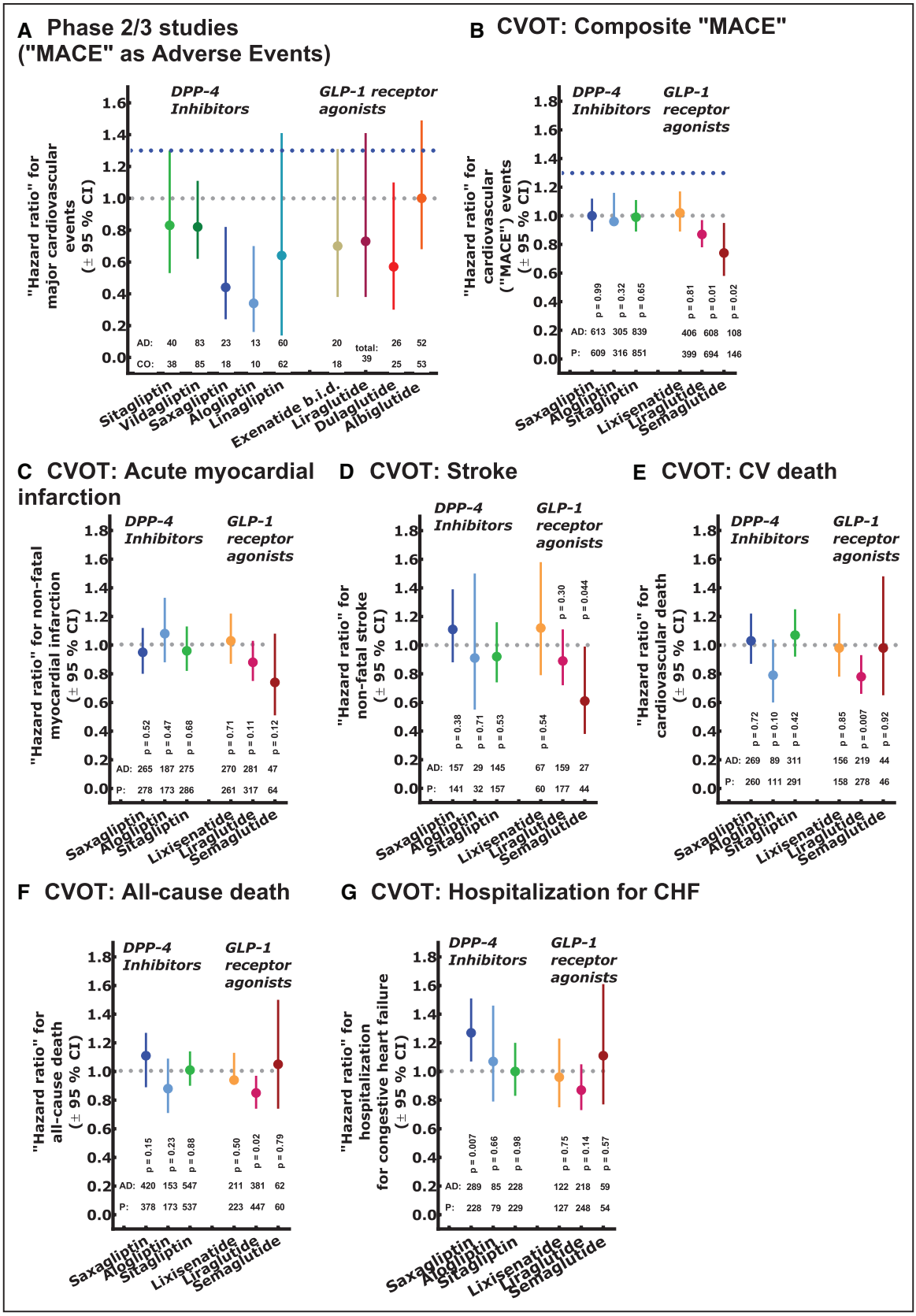

Figure 2. Hazard ratios (±95% confidence intervals [CIs]) for the time to first major adverse cardiovascular events versus placebo/comparator treatment.

A, Major cardiovascular adverse events (MACE) from meta-analyses of phase 2 and 3 clinical trials programs with dipeptidyl peptidase-4 inhibitors (DPP-4Is) (sitagliptin,[154] vildagliptin,[155] saxagliptin,[156] alogliptin,[157] and linagliptin[158]) and (*Continued*)

Blood Pressure

Systolic blood pressure (BP) may transiently rise with acute exposure to pharmacological but not physiological concentrations of (intravenous) GLP-1 or GLP-1RAs (Table 1). More sustained treatment with GLP-1RAs consistently leads to a reduction in systolic BP by 2 to 3 mm Hg in subjects with hypertension (Figure 2). Diastolic BP is less consistently affected. DPP-4Is have no uniform BP-lowering effect, but in patients with arterial hypertension, lowering of systolic BP has been reported.[52] Although a role for atrial natriuretic peptide leading to vasodilation and renal sodium excretion has been delineated in rodents,[111] the existence of a GLP-1R-ANF axis has not been uniformly replicated in human subjects with hypertension and T2D (Table 1),[73,76] suggesting that atrial natriuretic peptide secretion does not mediate the GLP-1-mediated reduction of BP in obese subjects or those with diabetes mellitus. A rise in atrial natriuretic peptide or brain natriuretic peptide levels has not been consistently reported with liraglutide[74,75] (Table 1), but a rise in atrial natriuretic peptide has been observed with exenatide treatment.[112] Nevertheless, acute or chronic GLP-1RAs promote natriuresis in healthy patients, obese patients, or those with diabetes mellitus.[6,76] Whether the magnitude of the natriuresis is sufficient to contribute to the reduction of BP and cardiovascular events is not clear. These decreases in BP may potentially be mediated by reductions in angiotensin II concentrations[113] or the local release of nitric oxide, which results in vasodilatation (Table 1).

Renal Function

Intravenous GLP-1 may acutely raise glomerular filtration rates,[78] whereas exenatide did not change glomerular filtration[79] (Table 1). Liraglutide significantly reduced albuminuria in short-term studies and in the LEADER trial.[7,74] Small reductions in albuminuria or favorable changes between the categories of micro- and microalbuminuria have also been described for the DPP-4Is saxagliptin[34,114]and linagliptin.[115] There have been no clinically significant changes in GFR or other renal events in randomized controlled trials of DPP-4 inhibition.

Metabolism

Apart from the well-known effects of GLP-1 and the incretin-based glucose-lowering medications on plasma glucose and HbA_{1c} levels (Figure 1), GLP-1 may transiently reduce the concentrations of nonesterified fatty acids.[82] Effects of incretin-based medications on fasting lipids and lipoproteins, most evident after weight loss, are shown in Figure 1. The most compelling effect of GLP-1, GLP-1RAs (exenatide, liraglutide), and DPP-4Is (sitagliptin, vildagliptin, alogliptin) has been a reduction in the postprandial rise in triglycerides and apolipoprotein-B 48, constituents of intestinally derived chylomicrons (Table 1). Tracer studies in healthy humans without diabetes mellitus treated acutely with sitagliptin confirm that the predominant mechanism linking DPP-4 inhibition to reduction of circulating chylomicrons is by attenuation of intestinal lipoprotein production.[116] Although acute administration of GLP-1 delays gastric emptying and slows intestinal nutrient delivery, exenatide still inhibited intestinal lipoprotein production and secretion after intraduodenal nutrient delivery in human subjects[84] (Table 1). Because DPP-4Is do not slow gastric emptying, the major effect of these agents is on the synthesis and secretion of chylomicrons.[116] Proatherosclerotic chylomicron remnant particles were also reduced with exenatide treatment.[84] Levels of postprandial triglycerides may contribute to atherosclerotic risk.[117]

Hepatic Triglyceride Deposition (Steatosis, Nonalcoholic Fatty Liver Disease)

Excessive hepatic triglyceride deposition has been linked to increased cardiovascular risk in subjects with T2D.[118] Exenatide[90] and liraglutide[91] may reverse fatty liver disease (by ultrasonography criteria) or steatohepatitis (based on histological criteria), respectively (for details and references, see Table 1). It is difficult to conclude whether the main mechanism reflects body weight loss or changes in glucose, lipids, or mediators of inflammation. Studies with DPP-4Is have shown a reduction in hepatic triglyceride content with vildagliptin[92] and no significant effects with sitagliptin.[93] The mechanisms are unknown because most studies do not support the expression of GLP-1R in human hepatocytes.

Inflammation

GLP-1[94] and the GLP-1RA exenatide[95] reduce the generation of reactive oxygen species in endothelial

Figure 2 (Continued). glucagon-like peptide-1 (GLP-1) receptor agonists (exenatide BID,[159] liraglutide,[160] dulaglutide,[161] and albiglutide[162]). (**B** through **G** concern results reported for CVOTs). **B,** MACE. **C,** Acute myocardial infarction. **D,** Stroke. **E,** Cardiovascular death. **F,** All-cause death. **G,** Hospitalization for congestive heart failure (CHF) from cardiovascular outcomes trials. CVOT results have been published for saxgliptin,[34] alogliptin,[36] and sitagliptin[33] (DPP-4Is); and for lixisenatide,[80] liraglutide,[7] and semaglutide[8] (GLP-1 receptor agonists). MACE analysis in CVOTs may include hospitalization for unstable angina; acute myocardial infarction has variably been reported as all (fatal and nonfatal) or only nonfatal cases; stroke has been reported as ischemic or all stroke. See definition of end points for individual outcomes trials in Table 2. BID indicates twice daily; CHF, congestive heart failure; CV, cardiovascular; and CVOT, cardiovascular outcome trial.

cells and monocytes/macrophages. Although less well studied, acute and chronic administration of sitagliptin also reduced molecular markers of inflammation in circulating mononuclear cells from human subjects with T2D.[97] Specifically, nuclear factor κB binding in mononucleocytes was reduced by the exenatide[95] and sitagliptin.[97] GLP-1, GLP-1RAs (exenatide, liraglutide), and a DPP-4I (sitagliptin) reduced the production of inflammatory cytokines such as tumor necrosis factor-α, interleukins 1ß and 6, and intercellular adhesion molecules (Table 1). Liraglutide reduced the expression of the inflammatory macrophage activation molecule sCD163 by 22%.[99] GLP-1RAs (exenatide, liraglutide) and a DPP-4Is (sitagliptin) also reduced levels of C-reactive protein (Table 1). Adiponectin, an adipocytokine that reduces insulin resistance, was increased by GLP-1RAs (exenatide and liraglutide) and DPP-4Is (sitagliptin and vildagliptin) (Table 1). Nevertheless, many of these studies are not controlled for changes in insulin or reductions in glycemia or body weight, and ascertainment of the underlying mechanisms coupling incretin therapy to reduced inflammation in humans is challenging.

Platelet Aggregation/Thrombosis

GLP-1r mRNA transcripts were detected in the human megakaryocyte MEG-1 cell line, which also exhibited functional cAMP accumulation in a dose-dependent manner in response to increasing concentrations of native GLP-1 and exenatide.[104] Whether isolated human platelets express a functional canonical GLP-1R has not yet been definitively demonstrated, although Steven and colleagues[119] reported immunoreactive GLP-1R protein in murine and human platelets by Western blot analysis using a commercial antibody. In contrast, studies examining the human platelet transcriptome have failed to consistently detect *GLP-1R* expression.[120] Although GLP-1 receptor stimulation may have the potential to reduce platelet aggregation, only preliminary studies in humans have addressed potential effects on the stability of atherosclerotic plaques in general and in coronary vessels in particular.[121]

Taken together, a large body of work demonstrates the effects of native GLP-1 and its degradation products, GLP-1RAs and DPP-4Is, in the cardiovascular system. These studies highlight potential mechanisms by which clinically meaningful modifications occur in the pathophysiology of coronary heart disease, acute myocardial infarction, congestive heart failure, and peripheral artery disease. The effects shown for GLP-1RAs and DPP-4Is appear to be qualitatively similar, whereas differences in effect sizes may occur. Comparative human studies characterizing quantitative differences between effects of GLP-1RAs and DPP-4Is are mostly lacking.

INFLUENCE OF GLP-1RAS AND DPP-4IS ON CARDIOVASCULAR RISK FACTORS

GLP-1RAs and DPP-4Is have been approved as glucose-lowering agents for subjects with T2D. GLP-1RAs reduce fasting glucose concentrations substantially (by on average, up to 50 mg/dL) and HbA_{1c} by 0.5 to 1.3%, whereas DPP-4Is typically reduce fasting glucose concentrations by on average, up to 25 mg/dL and HbA_{1c} by 0.6% to 0.9% (Figure 1). It is notable that GLP-1RAs with a short duration of action (achieving effective drug concentrations for less than a full 24-hour period) have smaller effects on fasting glucose and HbA_{1c} concentrations. Nevertheless, short-acting agents have more pronounced effects on gastric emptying and glycemic increments after a meal because they evade tachyphylaxis (for gastric emptying) typically induced by sustained exposure to high doses.[110] Because of the short-acting nature of lixisenatide leading to transient exposure to the drug after once-daily injection, effects on postprandial glycaemia are mainly present after the first meal following the subcutaneous injection of lixisenatide.

The glucose-dependent mechanism of modulating insulin and glucagon secretion confers incretin-based glucose-lowering medications with a low risk of hypoglycemia.[1] However, incretin therapies do not prevent hypoglycemic episodes prompted by other diabetes mellitus medications, especially when combined with sulfonylureas or insulin.[1,2] Nevertheless, DPP-4Is and GLP-1RAs have a lower risk for hypoglycemia when combined with sulfonylureas than is seen with insulin therapy.[144] Hypoglycemic episodes, especially severe hypoglycemic episodes (requiring third-party assistance for management) in population with T2D, are associated with increased risk of cardiovascular events and mortality.[145]

GLP-1RAs typically lead to reductions in appetite and food intake resulting in a loss in body weight (Figure 1). The reduction in body weight achieved is usually between 2 and 4 kg on average[146] but may be highly variable.[146] DPP-4Is may lead to minor reduction in body weight but usually by <1 kg.[1,2]

GLP-1 can acutely lower nonesterified fatty acid concentrations through the stimulation of insulin and suppression of glucagon secretion, especially in patients with T2D and hyperglycemia.[82] In clinical trials, GLP-1RAs have been shown to lower triglyceride concentrations and increase high-density lipoprotein cholesterol (ie, to ameliorate the dyslipidemia typically associated with insulin resistance) (Figure 1). Improvements in fasting triglycerides and high-density lipoprotein cholesterol may vary with the individual degree of weight reduction and glycemic control achieved but also between GLP-1RAs. In addition, a small but consistent reduction in low-density lipoprotein cholesterol concentrations has been observed with GLP-1RAs, especially when com-

paring this treatment modality to insulin regimens.[144] DPP-4Is do not have major effects on fasting lipoprotein patterns (Figure 1), although they do prevent or delay postprandial chylomicron formation, similar to findings with GLP-1RAs (Table 1).

CARDIOVASCULAR OUTCOMES TRIALS WITH INCRETIN-DERIVED GLUCOSE-LOWERING MEDICATIONS

Before 2008, the US Food and Drug Administration approved drugs used in the treatment of patients with diabetes mellitus based on their effectiveness in reducing blood glucose. Trials designed to demonstrate glucose-lowering efficacy were typically short (<1 year) trials in a relatively healthy population with few cardiovascular events and used HbA_{1c} as a surrogate end point. After results from trials with several glucose-lowering drugs were shown to possibly increase the risk of cardiovascular events,[147,148] the US Food and Drug Administration issued guidance in 2008 requiring new diabetes mellitus therapies to demonstrate cardiovascular safety (http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm071627.pdf). Henceforth, randomized clinical trials were designed to demonstrate that therapies did not significantly increase the risk of cardiovascular events. Following this guidance, trial results are now available for studies of numerous glucose-lowering therapies, including DPP-4Is (saxagliptin,[34] alogliptin,[36] sitagliptin[33]), GLP-1RAs (lixisenatide,[80] liraglutide,[7] semaglutide[8]), and sodium-glucose cotransporter-2 inhibitors (empagliflozin[142]).

The cardiovascular safety trials performed to date have included human subjects with T2D at increased risk of cardiovascular events. Thus, subjects enrolled in these trials had prior cardiovascular events or presented with risk factors for cardiovascular events. Although this was not an explicit inclusion criterion, typically they had longstanding T2D, and a substantial proportion was treated with insulin (Table 2). To maintain glycemic equipoise, patients have been typically randomized to the therapy of interest or matching placebo, and investigators have been encouraged to monitor the hemoglobin A_{1c} in all patients and adjust glucose-lowering therapies accordingly. As a result of standard care allowing or even requiring changes in the glucose-lowering treatment in line with applicable guidelines, cardiovascular outcome studies are not useful for comparing glucose-lowering effects of different medications.

Cardiovascular Outcomes Trials With DPP-4Is

Saxagliptin,[34] alogliptin,[36] and sitagliptin[33] have been studied in appropriately powered cardiovascular outcomes trials (SAVOR-TIMI 53, EXAMINE, TECOS). Although all cardiovascular outcomes trials listed in Table 2 describe patients at high risk for cardiovascular events, the inclusion criteria varied substantially from individuals with a recent acute coronary syndrome to those with a previous history of cardiovascular events to subjects with only risk factors present at baseline. Nevertheless, the results of these studies of DPP-4Is on major adverse cardiovascular events have been similar. Inhibition of DPP-4 neither increased nor decreased the risk of the combined major adverse cardiovascular event outcome (Figure 2B), which includes cardiovascular death (Figure 2E), myocardial infarction (Figure 2C), or stroke (Figure 2D), including or not including hospitalization for unstable angina in the composite end point. Thus, the cardiovascular safety of these agents has been confirmed (Figure 2B).

In the SAVOR-TIMI 53 trial, patients randomized to saxagliptin had a 0.7% absolute increase in the risk of hospitalization for heart failure (3.5% versus 2.8%; HR, 1.27; 95% CI, 1.07–1.51).[35] In the EXAMINE trial, a greater number of patients treated with alogliptin were hospitalized for heart failure, but this difference was not statistically significant (3.1% versus 2.9%; HR, 1.07; 95% CI, 0.79–1.46).[37] However, in the TECOS trial, no difference occurred in the rates of hospitalization for heart failure (3.1% versus 3.1%; HR, 1.00; 95% CI, 0.84–1.20)[163] (Figure 2G). Subsequently, a large observational study did not find evidence that DPP-4 inhibition with either saxagliptin or sitagliptin was associated with hospitalization for heart failure.[164] A meta-analysis summarizing the risk for hospitalization because of congestive heart failure in cardiovascular outcomes trials indicated a nonsignificant elevation in risk (HR, 1.14; 95% CI, 0.97–1.34; P=0.10),[163] with substantial heterogeneity between trials with saxagliptin,[34] alogliptin,[36] and sitagliptin.[33] The lack of evidence for an effect of sitagliptin on heart failure, relative to the findings with saxagliptin and possibly alogliptin, may reflect differences in trial design or unique attributes of each drug and remains unexplained.

Cardiovascular Outcomes Trials With GLP-1RAs

The cardiovascular safety of GLP-1RAs has been evaluated in several randomized clinical trials. Lixisenatide was studied in 6068 patients with recent hospitalization for acute coronary syndrome. Neither an increase nor a decrease occurred in the risk of cardiovascular events with lixisenatide therapy (Figure 2B).[80] However, in separate studies, the longer-acting GLP-1RAs liraglutide and semaglutide reduced numbers of cardiovascular events. In the LEADER trial, 9340 patients at high risk of cardiovascular events (81% with established cardiovascular disease) were randomized to either liraglutide or

Table 2. Patient Baseline Characteristics and Essential Protocol Details for Cardiovascular Outcomes Trials With Incretin-Based Therapies

	Medication Class											
	DPP-4Is						GLP-1 Receptor Agonists					
	SAVOR-TIMI 53[34,149] /saxagliptin		EXAMINE[36,150]/ alogliptin		TECOS[33,151] /sitagliptin		ELIXA[80,152] /lixisenatide		LEADER[7,153] /liraglutide		SUSTAIN-6[8] /semaglutide	
Characteristic	Active Drug	Placebo	Active Drug	Placebo	Active Drug	Placebo	Active Drug	Placebo	Active Drug	Placebo	Active Drug	Placebo
Administration	Oral	Oral	Oral	Oral	Oral	Oral	Subcutaneous	Subcutaneous	Subcutaneous	Subcutaneous	Subcutaneous	Subcutaneous
Dose, mg	5*	n.a.	25*	n.a.	100†	n.a.	20 µg	n.a.	1.78	n.a.	0.5/1.0	n.a.
Patient number, n	8280	8212	2701	2679	7332	7339	3034	3034	4668	4672	1648	1649
Sex, % female	33.4	32.7	32.3	32.0	29.1	29.5	30.4	30.9	35.5	36.0	38.5	40.0
Age, y	65±9	65±9	61†	61†	65±8	66±8	60±10	61±10	64±7	64±7	65±7	65±8
Body mass index, kg/m^2	31.1±5.5	31.2±5.7	28.7†	28.7†	30.2±5.6	30.2±5.7	30.1±5.6	30.2±5.8	32.5±6.3	32.5±6.3	32.8±6.3	32.8±6.1
Diabetes mellitus duration, y	10†	10†	7†	7†	12±8	12±8	9±8	9±8	13±8	13±8	14±8	14±8
HbA_{1c}, %	8.0±1.4	8.0±1.4	8.0±1.1	8.0±1.1	7.2±0.5	7.2±0.5	7.7±1.2	7.6±1.3	8.7±1.6	8.7±1.5	8.7±1.5	8.7±1.5
Insulin treatment, %	41.6	41.2	29.4	30.3	23.5	22.9	39.2	39.0	43.6	45.5	58.0	58.1
Median follow-up, y	2.1		1.5		3.0		2.1		3.8		2.1	
Premature drug discontinuation, %/y	8.8	9.9	13.9	15.1	8.7	9.2	13.1	11.4	n.r.	n.r.	10.1	9.0
eGFR	73±23	73±23	71.1†	71.2†	75±21	75±21	77±21	75±21	n.r.	n.r.	n.r.	n.r.
eGFR <60 mL/min, %	15.7‡	15.6‡	28.6	29.6	9.5‡	9.4‡	21.7	24.7	23.9	22.3	28.5	28.5
Prior cardiovascular disease, %	78.4	78.7	99.7	99.8	100.0	100.0	100.0	100.0	82.1	80.6	87.0‡‡	
Congestive heart failure, %	12.8	12.8	28.0	27.8	17.8	18.3	22.5	22.3	17.9	17.8	23.1	24.0
Statin use, %	78.3	78.4	90.6	90.3	79.8	80.0	93.3	92.2	72.7	71.4	72.8	72.8
Aspirin use, %	75.5	75.0	90.6¶	90.8¶	78.6	78.4	97.6¶	97.4¶	63.7	62.1	63.8	64.1
Patient selection criteria	Established cardiovascular disease or multiple risk factors‖		Recent ACS (within 15–90 days)**		Established cardiovascular disease‖		Recent acute coronary syndrome**		Age ≥50 y and established cardiovascular disease or age ≥60 y and risk factors		Age ≥50 y and established cardiovascular disease or age ≥60 y and risk factors	
Primary end point	Composite: cardiovascular death, myocardial infarction, ischemic stroke		Composite: cardiovascular death, nonfatal myocardial infarction, nonfatal stroke		Composite: cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, hospitalization for unstable angina		Composite: cardiovascular death, myocardial infarction stroke, hospitalization for unstable angina		Composite: cardiovascular death, nonfatal myocardial infarction, nonfatal stroke		Composite: cardiovascular death, nonfatal myocardial infarction, nonfatal stroke	
Cardiovascular events, n††	613	609	305	316	839	851	406	399	608	694	108	146

Values represent mean±standard deviation or proportion (%) displaying the characteristic in question. ACS indicates acute coronary syndrome; DPP-4Is, dipeptidyl peptidase-4; eGFR, estimated glomerular filtration rate; ELIXA, Evaluation of Lixisenatide in Acute Coronary Syndrome; EXAMINE, Examination of Cardiovascular Outcomes with Alogliptin versus Standard of Care; GLP-1, glucagon-like peptide-1; LEADER, Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results; n.a., not applicable; n.r., not reported; SAVOR-TIMI 53, Saxagliptin Assessment of Vascular Outcomes Recorded in Patients with Diabetes Mellitus-Thrombolysis in Myocardial Infarction; SUSTAIN-6, Trial to Evaluate Cardiovascular and Other Long-term Outcomes With Semaglutide in Subjects With Type 2 Diabetes; and TECOS, Trial to Evaluate Cardiovascular Outcomes after Treatment with Sitagliptin.

*Reduced dose in patients with chronic kidney disease.

†Median.

‡<50 mL/min.

¶Including other platelet aggregation inhibitors.

‖Major coronary artery disease, ischemic cerebrovascular disease, or atherosclerotic peripheral arterial disease.

**Acute myocardial infarction or unstable angina.

††As defined by the respective primary end point.

‡‡Only stated for the whole cohort.

placebo. Liraglutide reduced the risk for the combined primary outcome of cardiovascular death, myocardial infarction, or stroke by 13% (13.0% versus 14.9%; HR, 0.87; 95% CI, 0.78–0.97). There were directionally consistent effects on cardiovascular death (4.7% versus 6.0%; HR, 0.78; 95% CI, 0.66–0.93), myocardial infarction (HR, 0.86; 95% CI, 0.73–1.00; *P*=0.046), and stroke (HR, 0.86; 95% CI, 0.71–1.06) (Figure 2).

No significant difference in rates of hospitalization for heart failure occurred with liraglutide versus placebo in the LEADER trial (4.7% versus 5.3%; HR, 0.87; 95% CI, 0.73–1.05). These results are consistent with findings from a study in patients with more severe heart failure (baseline left ventricular ejection fraction ≤40%), which showed treatment with liraglutide over 180 days did not significantly change a mean global rank score evaluating time to death or hospitalization because of heart failure and changes in N-terminal pro-brain natriuretic peptide.[30,165] However, the rates of hospitalization for heart failure in the patients treated with liraglutide in this study were numerically greater but not statistically different (41% [liraglutide] versus 34% [placebo]; HR, 1.30; 95% CI, 0.89–1.88).

Evidence for the cardiovascular benefit of GLP-1RAs was also seen with semaglutide, an investigational long-acting, once-weekly GLP-1RA, in the SUSTAIN-6 trial.[8] This trial was designed as a noninferiority safety study and randomized 3297 patients with diabetes mellitus at high risk of cardiovascular disease (61% with established coronary artery disease). Treatment with semaglutide decreased the combined primary outcome of time to cardiovascular disease death, nonfatal myocardial infarction, or nonfatal stroke (6.6% versus 8.9%; HR, 0.74; 95% CI, 0.58–0.95). There was no difference in cardiovascular disease death (2.7% versus 2.8%; HR, 0.98; 95% CI, 0.65–1.48), and the reduction in the primary end point was driven by differences in myocardial infarction (2.9% versus 3.9%; HR, 0.74; 95% CI, 0.51–1.08) and stroke (1.6% versus 2.7%; HR, 0.61; 95% CI, 0.38–0.99). No statistically significant differences occurred in hospitalization for heart failure with semaglutide (3.6% [semaglutide] versus 3.3% [placebo]; HR, 1.11; 95% CI, 0.77–1.61) (Figure 2).

Thus, these trials have demonstrated cardiovascular safety for GLP-1RAs. Two studies have shown significant reductions in cardiovascular events with liraglutide[7] and semaglutide[8] of 13% and 26%, respectively, whereas treatment with lixisenatide did not reduce cardiovascular events. The different results of these studies may reflect the unique pharmacology of the respective agents. For example, lixisenatide has a shorter duration of action than liraglutide and semaglutide.[110] Alternatively, differences in study design may be contributing. The ELIXA trial included only patients with a recent acute coronary syndrome, a condition associated with a particularly high risk for subsequent cardiovascular events. Differences in study duration, patient risk profile, and study size may also influence trial-specific findings. Further studies, ideally head-to-head studies of GLP-1RAs, are needed to determine whether meaningful differences might explain these disparate findings.

WHAT MEDIATES BENEFICIAL CARDIOVASCULAR EFFECTS OF THE GLP-1RAS LIRAGLUTIDE AND SEMAGLUTIDE?

Metabolic Results From Trials With GLP-1RAs

Liraglutide and semaglutide were developed as glucose-lowering medications for the treatment of patients with T2D. Semaglutide has not yet been approved. However, these agents also reduce body weight, systolic blood pressure, and, in some patients, triglyceride and low-density lipoprotein cholesterol.[1,2] Thus, although some have assumed that the cardiovascular effects found in the LEADER trial[7] and the SUSTAIN-6 trial[8] reflect differences in glucose/HbA_{1c}, it seems more likely that the reductions in cardiovascular events with these agents are driven by nonglycemic effects of these drugs. Liraglutide[7] and semaglutide[8] displayed the largest difference, relative to patients treated with placebos, in glycohemoglobin. The difference amounted to 0.40% (95% CI, 0.34–0.45) at 36 months but was larger (7.2% versus 8.2% with placebo) at 3 months with liraglutide.[7] With semaglutide, glycohemoglobin was lower by 0.66% (95% CI, 0.52–0.88) with the 0.5 mg dose and by 1.05% (95% CI, 0.91–1.19) with 1 mg per week.[8] This finding contrasts with the much smaller reduction in HbA_{1c} with lixisenatide (−0.27% [95% CI, −0.31 to −0.22]), although after 12 weeks this difference was larger (0.4%).[80] Small but significant weight loss occurred with lixisenatide (−0.7 [95% CI, −0.9 to −0.5] kg),[80] and more substantial weight loss occurred with liraglutide (−2.3 [95% CI, −2.5 to −2.0] kg)[7] and semaglutide (0.5 mg: −2.9 [−3.5 to −2.3] kg; 1.0 mg: −4.4 [−4.1 to −3.8] kg).[8] Along the same lines, systolic BP was slightly reduced by lixisenatide (−0.8 [95% CI, −1.3 to −0.3] mm Hg)[80] but more substantially so by liraglutide (Δ −1.2 [95% CI, −1.9 to −0.5] mm Hg)[7] and semaglutide (0.5 mg: Δ 1.3 [95% CI, −2.8 to 1.2] mm Hg; 1.0 mg: −2.6 [−4.1 to −1.1] mm Hg).[8] Thus, significant reductions in cardiovascular events occurred with those treatments that induced the largest reductions in HbA_{1c}, body weight, and systolic BP. In addition, with liraglutide, a reduced risk for hypoglycemic episodes occurred, including severe hypoglycemic episodes.[7] This trend was observed with lixisenatide[80] but not with semaglutide.[8] Severe hypoglycemic episodes in patients with T2D have been linked to adverse cardiovascular and mortality outcomes.[145]

Metabolic Results From Trials With DPP-4Is

In cardiovascular outcomes trials with the DPP-4Is saxagliptin,[34] alogliptin,[36] and sitagliptin,[33] differences in HbA_{1c} of <0.4% were uniformly observed because of the addition of glucose-lowering medications added as part of standard care in all patients. Furthermore, there was little difference in body weight, BP, and heart rate between patients treated with DPP-4Is and placebos.

Other Glucose-Lowering Medications Introduced as Part of Standard Care

Because liraglutide and semaglutide are potent glucose-lowering agents and baseline HbA_{1c} concentrations in the LEADER[7] and SUSTAIN-6[8] trials were higher than common glycemic targets, additional glucose-lowering medications were added, more so in patients treated with placebo than in those treated with GLP-1RAs. Among those additional glucose-lowering medications were sulfonylureas and insulin. Observational studies have raised questions regarding the cardiovascular safety of sulfonylureas and insulin in patients at substantial risk for cardiovascular events. The propensity for these classes of glucose-lowering drugs to elicit (severe) hypoglycemic episodes is an additional concern. Further analyses of these outcomes trials may help indicate whether these added drugs may play a role in explaining the cardiovascular results.

Immediate Cardiovascular Effects of Stimulating GLP-1Rs

In meta-analyses of glucose-lowering trials, a significant reduction (by ≈15%) in the risk for acute myocardial infarction was shown when comparing intensive versus standard strategies for lowering glucose (representing an ≈0.9% difference in HbA_{1c} maintained over several years).[3,166] There was no effect on stroke or (cardiovascular) death within the core duration of such trials.[3,167] In some studies, a longer period of observation (>8 years) extending beyond the core trial period was necessary to show improvements in mortality,[168,169] whereas in the LEADER trial, there was a significantly reduced cardiovascular and all-cause mortality within a shorter period of time.[7] When analyzing the combined results of glucose-lowering trials, there was more significant benefit for subjects with shorter diabetes mellitus duration, better glycemic control, and no cardiovascular diagnosis at baseline.[167] In contrast, in the LEADER and SUSTAIN-6 trials, patients with preexisting cardiovascular disease displayed trends toward a greater benefit from liraglutide[7] and semaglutide[8] treatment, compared with those patients with only baseline risk factors, although these differences were not significant. In addition, the magnitude of changes in glycohemoglobin, body weight, and systolic BP elicited by liraglutide and semaglutide were smaller and maintained for shorter periods of time than would be predicted to be sufficient to elicit cardiovascular benefits based on previous analyses of glucose-lowering strategies.[3,167] The beneficial effects seen with liraglutide[7] and semaglutide[8] are consistent with diminishing atherogenic risk over time. The favorable within-trial changes seen in multiple cardiovascular risk factors (glycemia, body weight, systolic BP, lipids) (see Figure 1) may provide a conventional explanation for the results observed. However, previous analyses of glucose-lowering trials showed only a moderate reduction in myocardial infarction events with no change in (cardiovascular) mortality,[3] and even multifactorial interventions addressing BP (intensive management with antihypertensive medications), cholesterol (statins), and triglycerides (fibrates) reduced cardiovascular events, but not mortality[169] within a time frame similar to the duration of the cardiovascular outcomes trials with liraglutide (3.5 to 5 years)[7] and semaglutide (2 years).[8] Thus, it appears likely that traditional risk factor modification alone cannot explain the overall benefits observed, but rather additional mechanisms may be operative, most likely including direct effects in the cardiovascular system (Table 1). The reductions in nonfatal acute myocardial infarction and nonfatal stroke with both liraglutide and semaglutide (Figure 2) suggest a reduction of diabetes mellitus-related atherosclerotic disease, perhaps by reducing the plaque burden or increasing plaque stability. To date, however, no convincing evidence points to a single possible mechanism listed in Table 1 as playing a dominant role in the reduction of major adverse cardiovascular outcomes. Other potential mechanisms, such as reduced infarction-related myocardial necrosis (Figure I in the online-only Data Supplement) with liraglutide or semaglutide treatment remain to be demonstrated. More careful characterization of the expression and function of GLP-1Rs in the cardiovascular system in patients with diabetes mellitus, coronary artery disease, or congestive heart failure seems prudent. Potential influences of GLP-1RAs on cardiovascular risk and function are depicted in Figure 3.

Based on mechanistic findings in animals (Table I in the online-only Data Supplement) and human subjects with and without diabetes mellitus (Table 1), a beneficial effect of incretin-based medications on congestive heart failure was predicted, whereas only a (nonsignificant) trend for a reduction in the rate of hospitalization for congestive heart failure was observed in the LEADER trial,[7] with no similar observations from the other GLP-1RA trials, and some adverse outcomes from dedicated congestive heart failure trials with liraglutide[30] and detrimental findings with saxagliptin (DPP-4 I).[34,35] No widely accepted explanation for this

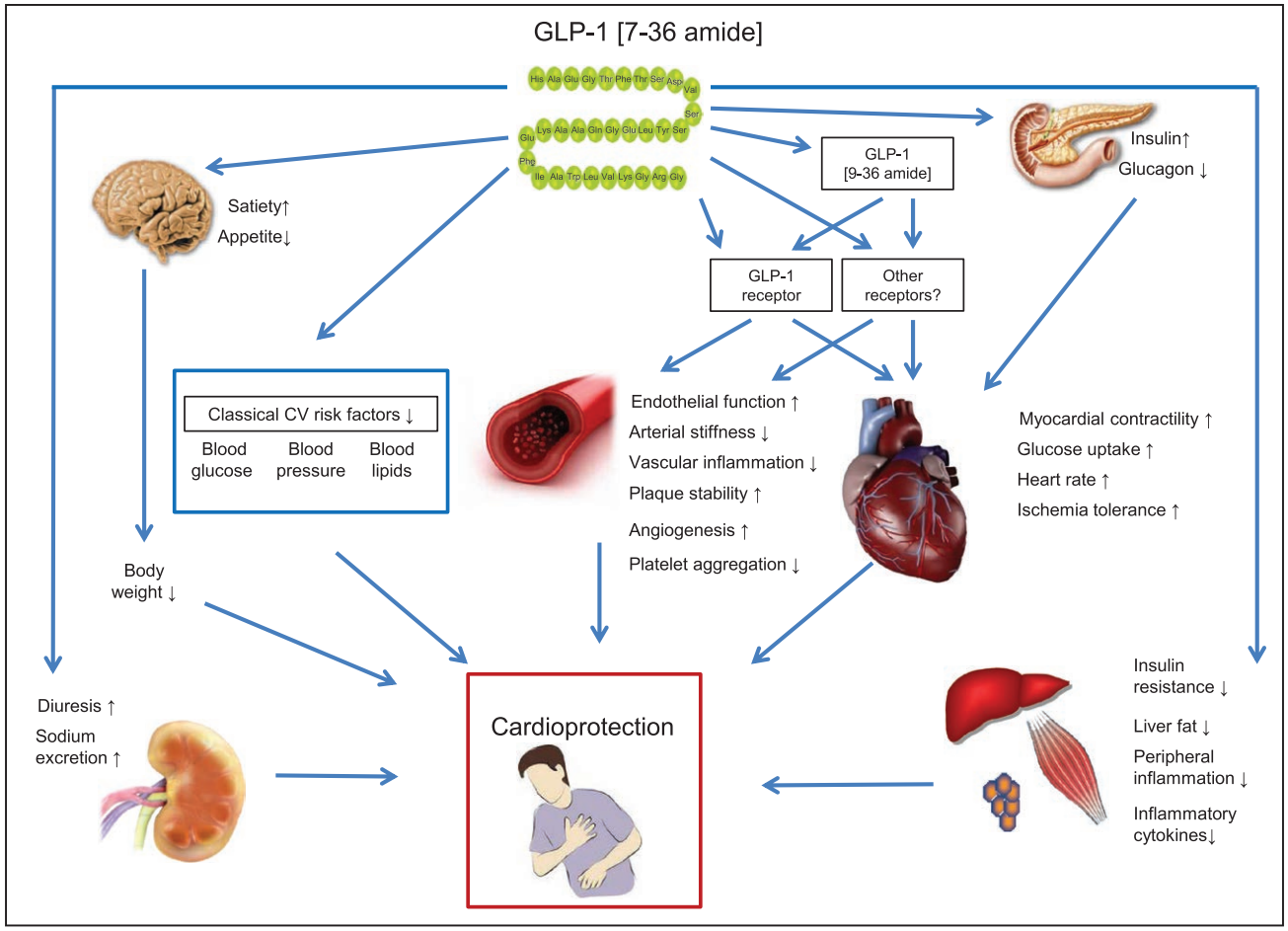

Figure 3. Potential mechanisms mediating a beneficial effect of glucagon-like peptide-1 (GLP-1) receptor agonists on reducing cardiovascular events.

Effects of diabetes mellitus-related parameters (glycemic control, avoidance of [severe] hypoglycemia), cardiovascular risk factors (body weight, blood pressure, lipoproteins/lipids), and interactions with GLP-1 receptors in the cardiovascular system (potentially leading to improved endothelial function/vasodilation, improved cardiac function under conditions of coronary ischemia, and anti-inflammatory/ anti-atherosclerotic effects) have to be considered. CV indicates cardiovascular.

discrepancy exists. Changes in the pattern of GLP-1R expression accompanying the development of cardiac ischemia or left ventricular failure or enhanced susceptibility to tachyarrhythmias might offer potential explanations. More studies addressing these possibilities are needed.

In contrast, the multitude of beneficial effects attributed to DPP-4 inhibition in mechanistic studies (Table 1) cannot easily be reconciled with the neutral results of large-scale clinical trials (Table 2, Figure 2). At this moment, no convincing explanation for this discrepancy exists. One possible explanation may reside in a different profile of potential DPP-4 substrates regulated by DPP-4Is in older humans with diabetes mellitus versus young animals with cardiovascular disease. A similar reasoning might explain potential mechanisms leading to worsening of congestive heart failure in some humans with DPP-4 inhibition.[34,35] However, no specific candidate or differential profile of DPP-4 peptide(s) substrates has been identified that would provide a satisfactory explanation. Furthermore, it is similarly difficult to explain why some specific agents belonging to the DPP-4I class display an increased risk for hospitalization for congestive heart failure, whereas others seem to be free of such a risk.

CLINICAL PERSPECTIVE

The clinical implications dictate that long-acting GLP-1RAs such as liraglutide (approved) and semaglutide (not yet approved) with demonstrated cardiovascular benefit should be used preferentially in human subjects matching the inclusion criteria of these cardiovascular outcomes trials. Their prescriptions should be weighed against that of empagliflozin (sodium-glucose cotransporter-1 inhibitor), which has shown benefits in terms of reducing (cardiovascular) mortality and hospitalization for congestive heart failure in similar populations of patients with T2D at high risk for cardiovascular

events.[170] Information on cardiovascular outcomes with a combination treatment of GLP-1RAs and sodium-glucose cotransporter-2 inhibitors is not available.

In contrast, the cardiovascular safety of DPP-4Is has uniformly been underscored by neutral findings in cardiovascular outcomes trials with saxagliptin, alogliptin, and sitagliptin. Findings of an increased risk for hospitalization because of congestive heart failure with the DPP-4Is saxagliptin (significant) and alogliptin (nonsignificant trend) in the absence of clear benefits regarding overall cardiovascular risk require further mechanistic clarification and caution in using these drugs in individuals at risk for congestive heart failure.

OUTLOOK

Evidence that 2 GLP-1RAs, liraglutide and semaglutide, reduce major adverse cardiovascular events in subjects with T2D at high risk for such complications (Figure 2) represents a major therapeutic advance. With these and additional glucose-lowering medications such as empagliflozin having a major impact on the risks for cardiovascular events and mortality, individualized treatment can be offered to patients with diabetes mellitus at risk for cardiovascular events. Whether there is a similar benefit for individuals at earlier stages of T2D with lower cardiovascular risk is not yet known. Continued research is needed to better define the mechanisms leading to the benefits seen to date (Table 3). It remains possible that the reductions in cardiovascular events seen in these trials are a direct result of GLP-1R signaling, given the numerous actions within the heart, blood vessels, and other tissues that impact risk factors (Table 1, Figure 1). Irrespective of the underlying mechanisms, the neutral (in the case of DPP-4Is) and even beneficial (in the case of long-acting GLP-1RAs), cardiovascular actions of the incretin-based glucose-lowering medications have broadened our armamentarium, enabling application of personalized medicine to reduce the detrimental complications of T2D.

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DISCLOSURES

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Table 3. Questions to Address in Future Research on the Effects of GLP-1, GLP-1 Receptor Agonists, and DPP-4Is in the Cardiovascular System

No.	Question
1	Where exactly are GLP-1 receptors expressed in the heart, in blood vessels, and in the autonomic and central nervous system (controlling cardiovascular functions)?
2	How can effects of the DPP-4-generated metabolite (GLP-1 [9–36 amide] or [9–37]) in the cardiovascular system be explained (not high-affinity agonists at the canonical GLP-1 receptor): Second receptor? Nonreceptor-mediated effects on adenylate cyclase? Mitochondria? Other mechanisms?
3	Why do DPP-4Is demonstrate a multitude of promising cardiovascular effects in mechanistic studies or short-term interventions but do not elicit cardiovascular benefit in outcomes studies?
4	Does cardiovascular disease change the expression or function of GLP-1 receptors (potentially promoting or preventing benefits under certain conditions)?
5	Are beneficial effects of GLP-1 receptor agonists mainly mediated by (1) improved glycemic control, (2) improvements in cardiovascular risk factors, or (3) direct interactions with GLP-1 receptors controlling cardiovascular function?
6	Will patient populations other than subjects with type 2 diabetes mellitus and preexisting cardiovascular damage display a similar cardiovascular benefit (eg, subjects with type 2 diabetes mellitus at lower cardiovascular risk, obese subjects without diabetes mellitus, subjects without diabetes mellitus in more general terms)?
7	How does the dose–response relationship for GLP-1 receptor agonists and cardiovascular effects compare to that for metabolic actions?
8	Can beneficial cardiovascular effects of stimulating GLP-1 receptors be used to treat conditions such as acute coronary syndrome and periprocedural glycemic control with cardiovascular and cardiosurgery interventions?
9	Which properties of GLP-1 receptor agonists (pharmacokinetics or ability to interact with cardiovascular GLP-1 receptors) are relevant for inducing cardiovascular benefits with liraglutide or semaglutide vs lixisenatide?
10	Why and how do some but not all DPP-4Is increase the risk of hospitalization for heart failure?

DPP-4I indicates dipeptidyl peptidase-4; and GLP-1, glucagon-like peptide-1.

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Literature search strategy: PubMed was screened using EndNote X7 combining search terms for the active agent (GLP-1, glucagon-like, exenatide, lixisenatide, liraglutide, dulaglutide, albiglutide, dipeptidyl peptidase, DPP-4, sitagliptin, vildagliptin, saxagliptin, alogliptin, linagliptin) with search terms indicating potential effects (myocardial, left ventircular, cardioprotection, ischemia, stunning, heart rate, angiogenesis, proliferation, endothelial/endothelium, vasodilation, nitric oxide, atherosclerosis/atherosclerotic, blood pressure, natriuretic peptide(s), atrial natriuretic peptide, brain natriuretic peptide, renal, glomerular, albumin/albuminuria, glucose, hyperglycemia, glycated hemoglobin, HbA_{1c}, body weight, body mass index, lipids, lipoproteins, triglycerides, chylomicrons, remnants, fatty acids, liver, hepatic, steatosis, fatty liver, nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, oxygen/oxidative, nuclear factor, inflammation/inflammatory, cytokine(s), C-reactive protein, adiponectin, interleukin(s), platelet, aggregation). In addition, reference lists of retrieved articles were screened.

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FOOTNOTES

The online-only Data Supplement is available with this article at http://circ.ahajournals.org/lookup/suppl/doi:10.1161/CIRCULATIONAHA.117.028136/-/DC1.

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REFERENCES

1. Drucker DJ, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet*. 2006;368:1696–1705.
2. Nauck M. Incretin therapies: highlighting common features and differences in the modes of action of glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors. *Diabetes Obes Metab*. 2016;18:203–216.
3. Ray KK, Seshasai SR, Wijesuriya S, Sivakumaran R, Nethercott S, Preiss D, Erqou S, Sattar N. Effect of intensive control of glucose on cardiovascular outcomes and death in patients with diabetes mellitus: a meta-analysis of randomised controlled trials. *Lancet*. 2009;373:1765–1772. doi: 10.1016/S0140-6736(09)60697-8.
4. Ussher JR, Drucker DJ. Cardiovascular actions of incretin-based therapies. *Circ Res*. 2014;114:1788–1803. doi: 10.1161/CIRCRESAHA.114.301958.
5. Ussher JR, Drucker DJ. Cardiovascular biology of the incretin system. *Endocr Rev*. 2012;33:187–215. doi: 10.1210/er.2011-1052.
6. Drucker DJ. The cardiovascular biology of glucagon-like peptide-1. *Cell Metab*. 2016;24:15–30. doi: 10.1016/j.cmet.2016.06.009.
7. Marso SP, Daniels GH, Brown-Frandsen K, Kristensen P, Mann JF, Nauck MA, Nissen SE, Pocock S, Poulter NR, Ravn LS, Steinberg WM, Stockner M, Zinman B, Bergenstal RM, Buse JB; LEADER Steering Committee; LEADER Trial Investigators. Liraglutide and cardiovascular outcomes in type 2 diabetes. *N Engl J Med*. 2016;375:311–322. doi: 10.1056/NEJMoa1603827.
8. Marso SP, Bain SC, Consoli A, Eliaschewitz FG, Jódar E, Leiter LA, Lingvay I, Rosenstock J, Seufert J, Warren ML, Woo V, Hansen O, Holst AG, Pettersson J, Vilsbøll T; SUSTAIN-6 Investigators. Semaglutide and cardiovascular outcomes in patients with type 2 diabetes. *N Engl J Med*. 2016;375:1834–1844. doi: 10.1056/NEJMoa1607141.
9. Drucker DJ. Deciphering metabolic messages from the gut drives therapeutic innovation: the 2014 Banting Lecture. *Diabetes*. 2015;64:317–326. doi: 10.2337/db14-1514.
10. Pujadas G, Drucker DJ. Vascular biology of glucagon receptor superfamily peptides: complexity, controversy, and clinical relevance. *Endocr Rev*. 2016:er20161078.
11. Giacco F, Du X, Carratú A, Gerfen GJ, D'Apolito M, Giardino I, Rasola A, Marin O, Divakaruni AS, Murphy AN, Shah MS, Brownlee M. GLP-1 cleavage product reverses persistent ROS generation after transient hyperglycemia by disrupting an ROS-generating feedback loop. *Diabetes*. 2015;64:3273–3284. doi: 10.2337/db15-0084.
12. Mundil D, Beca S, Cameron-Vendriug A, El-Mounayri O, Momen A, Backx PH, Husain M. GLP-1 [28–36] exerts direct cardioprotective effects, activating pro-survival kinases and soluble adenylyl cyclase (abstract 13657). *Circulation*. 2012;126 (suppl 21):A13657.
13. Mulvihill EE, Drucker DJ. Pharmacology, physiology, and mechanisms of action of dipeptidyl peptidase-4 inhibitors. *Endocr Rev*. 2014;35:992–1019. doi: 10.1210/er.2014-1035.
14. Bose AK, Mocanu MM, Carr RD, Brand CL, Yellon DM. Glucagon-like peptide 1 can directly protect the heart against ischemia/reperfusion injury. *Diabetes*. 2005;54:146–151.
15. DeNicola M, Du J, Wang Z, Yano N, Zhang L, Wang Y, Qin G, Zhuang S, Zhao TC. Stimulation of glucagon-like peptide-1 receptor through exendin-4 preserves myocardial performance and prevents cardiac remodeling in infarcted myocardium. *Am J Physiol Endocrinol Metab*. 2014;307:E630–E643. doi: 10.1152/ajpendo.00109.2014.
16. Wohlfart P, Linz W, Hübschle T, Linz D, Huber J, Hess S, Crowther D, Werner U, Ruetten H. Cardioprotective effects of lixisenatide in rat myocardial ischemia-reperfusion injury studies. *J Transl Med*. 2013;11:84. doi: 10.1186/1479-5876-11-84.
17. Bao W, Aravindhan K, Alsaid H, Chendrimada T, Szapacs M, Citerone DR, Harpel MR, Willette RN, Lepore JJ, Jucker BM. Albiglutide, a long lasting glucagon-like peptide-1 analog, protects the rat heart against ischemia/reperfusion injury: evidence for improving cardiac metabolic efficiency. *PLoS One*. 2011;6:e23570. doi: 10.1371/journal.pone.0023570.
18. Chua S, Lee FY, Tsai TH, Sheu JJ, Leu S, Sun CK, Chen YL, Chang HW, Chai HT, Liu CF, Lu HI, Yip HK. Inhibition of dipeptidyl peptidase-IV enzyme activity protects against myocardial ischemia-reperfusion injury in rats. *J Transl Med*. 2014;12:357. doi: 10.1186/s12967-014-0357-0.
19. Hocher B, Sharkovska Y, Mark M, Klein T, Pfab T. The novel DPP-4 inhibitors linagliptin and BI 14361 reduce infarct size after myocardial ischemia/reperfusion in rats. *Int J Cardiol*. 2013;167:87–93. doi: 10.1016/j.ijcard.2011.12.007.
20. Kristensen J, Mortensen UM, Schmidt M, Nielsen PH, Nielsen TT, Maeng M. Lack of cardioprotection from subcutaneously and preischemic administered liraglutide in a closed chest porcine ischemia reperfusion model. *BMC Cardiovasc Disord*. 2009;9:31. doi: 10.1186/1471-2261-9-31.
21. Pyke C, Heller RS, Kirk RK, Ørskov C, Reedtz-Runge S, Kaastrup P, Hvelplund A, Bardram L, Calatayud D, Knudsen LB. GLP-1 receptor localization

in monkey and human tissue: novel distribution revealed with extensively validated monoclonal antibody. *Endocrinology*. 2014;155:1280–1290. doi: 10.1210/en.2013-1934.
22. Gejl M, Lerche S, Mengel A, Møller N, Bibby BM, Smidt K, Brock B, Søndergaard H, Bøtker HE, Gjedde A, Holst JJ, Hansen SB, Rungby J. Influence of GLP-1 on myocardial glucose metabolism in healthy men during normo- or hypoglycemia. *PLoS One*. 2014;9:e83758. doi: 10.1371/journal.pone.0083758.
23. Gejl M, Søndergaard HM, Stecher C, Bibby BM, Møller N, Bøtker HE, Hansen SB, Gjedde A, Rungby J, Brock B. Exenatide alters myocardial glucose transport and uptake depending on insulin resistance and increases myocardial blood flow in patients with type 2 diabetes. *J Clin Endocrinol Metab*. 2012;97:E1165–E1169. doi: 10.1210/jc.2011-3456.
24. Witteles RM, Keu KV, Quon A, Tavana H, Fowler MB. Dipeptidyl peptidase 4 inhibition increases myocardial glucose uptake in nonischemic cardiomyopathy. *J Card Fail*. 2012;18:804–809. doi: 10.1016/j.cardfail.2012.07.009.
25. Sokos GG, Nikolaidis LA, Mankad S, Elahi D, Shannon RP. Glucagon-like peptide-1 infusion improves left ventricular ejection fraction and functional status in patients with chronic heart failure. *J Card Fail*. 2006;12:694–699. doi: 10.1016/j.cardfail.2006.08.211.
26. Halbirk M, Nørrelund H, Møller N, Holst JJ, Schmitz O, Nielsen R, Nielsen-Kudsk JE, Nielsen SS, Nielsen TT, Eiskjaer H, Bøtker HE, Wiggers H. Cardiovascular and metabolic effects of 48-h glucagon-like peptide-1 infusion in compensated chronic patients with heart failure. *Am J Physiol Heart Circ Physiol*. 2010;298:H1096–H1102. doi: 10.1152/ajpheart.00930.2009.
27. Wallner M, Kolesnik E, Ablasser K, Khafaga M, Wakula P, Ljubojevic S, Thon-Gutschi EM, Sourij H, Kapl M, Edmunds NJ, Kuzmiski JB, Griffith DA, Knez I, Pieske B, von Lewinski D. Exenatide exerts a PKA-dependent positive inotropic effect in human atrial myocardium: GLP-1R mediated effects in human myocardium. *J Mol Cell Cardiol*. 2015;89(Pt B):365–375. doi: 10.1016/j.yjmcc.2015.09.018.
28. Nathanson D, Frick M, Ullman B, Nyström T. Exenatide infusion decreases atrial natriuretic peptide levels by reducing cardiac filling pressures in type 2 diabetes patients with decompensated congestive heart failure. *Diabetol Metab Syndr*. 2016;8:5. doi: 10.1186/s13098-015-0116-2.
29. Lepore JJ, Olson E, Demopoulos L, Haws T, Fang Z, Barbour AM, Fossler M, Davila-Roman VG, Russell SD, Gropler RJ. Effects of the novel long-acting GLP-1 agonist, albiglutide, on cardiac function, cardiac metabolism, and exercise capacity in patients with chronic heart failure and reduced ejection fraction. *JAMA Heart Fail*. 2016;4:559–566. doi: 10.1016/j.jchf.2016.01.008.
30. Margulies KB, Hernandez AF, Redfield MM, Givertz MM, Oliveira GH, Cole R, Mann DL, Whellan DJ, Kiernan MS, Felker GM, McNulty SE, Anstrom KJ, Shah MR, Braunwald E, Cappola TP; NHLBI Heart Failure Clinical Research Network. Effects of liraglutide on clinical stability among patients with advanced heart failure and reduced ejection fraction: a randomized clinical trial. *JAMA*. 2016;316:500–508. doi: 10.1001/jama.2016.10260.
31. Jorsal A, Kistorp C, Holmager P, Tougaard RS, Nielsen R, Hänselmann A, Nilsson B, Møller JE, Hjort J, Rasmussen J, Boesgaard TW, Schou M, Videbaek L, Gustafsson I, Flyvbjerg A, Wiggers H, Tarnow L. Effect of liraglutide, a glucagon-like peptide-1 analogue, on left ventricular function in stable chronic heart failure patients with and without diabetes (LIVE): a multicentre, double-blind, randomised, placebo-controlled trial. *Eur J Heart Fail*. 2017;19:69–77. doi: 10.1002/ejhf.657.
32. Nogueira KC, Furtado M, Fukui RT, Correia MR, Dos Santos RF, Andrade JL, Rossi da Silva ME. Left ventricular diastolic function in patients with type 2 diabetes treated with a dipeptidyl peptidase-4 inhibitor- a pilot study. *Diabetol Metab Syndr*. 2014;6:103. doi: 10.1186/1758-5996-6-103.
33. Green JB, Bethel MA, Armstrong PW, Buse JB, Engel SS, Garg J, Josse R, Kaufman KD, Koglin J, Korn S, Lachin JM, McGuire DK, Pencina MJ, Standl E, Stein PP, Suryawanshi S, Van de Werf F, Peterson ED, Holman RR; Group TS. Effect of sitagliptin on cardiovascular outcomes in type 2 diabetes. *N Engl J Med*. 2015;373:232–242.
34. Scirica BM, Bhatt DL, Braunwald E, Steg PG, Davidson J, Hirshberg B, Ohman P, Frederich R, Wiviott SD, Hoffman EB, Cavender MA, Udell JA, Desai NR, Mosenzon O, McGuire DK, Ray KK, Leiter LA, Raz I; SAVOR-TIMI 53 Steering Committee and Investigators. Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. *N Engl J Med*. 2013;369:1317–1326. doi: 10.1056/NEJMoa1307684.
35. Scirica BM, Braunwald E, Raz I, Cavender MA, Morrow DA, Jarolim P, Udell JA, Mosenzon O, Im K, Umez-Eronini AA, Pollack PS, Hirshberg B, Frederich R, Lewis BS, McGuire DK, Davidson J, Steg PG, Bhatt DL; SAVOR-TIMI 53 Steering Committee and Investigators. Heart failure, saxagliptin, and diabetes mellitus: observations from the SAVOR-TIMI 53 randomized trial. *Circulation*. 2014;130:1579–1588. doi: 10.1161/CIRCULATIONAHA.114.010389.
36. White WB, Cannon CP, Heller SR, Nissen SE, Bergenstal RM, Bakris GL, Perez AT, Fleck PR, Mehta CR, Kupfer S, Wilson C, Cushman WC, Zannad F; EXAMINE Investigators. Alogliptin after acute coronary syndrome in patients with type 2 diabetes. *N Engl J Med*. 2013;369:1327–1335. doi: 10.1056/NEJMoa1305889.
37. Zannad F, Cannon CP, Cushman WC, Bakris GL, Menon V, Perez AT, Fleck PR, Mehta CR, Kupfer S, Wilson C, Lam H, White WB; EXAMINE Investigators. Heart failure and mortality outcomes in patients with type 2 diabetes taking alogliptin versus placebo in EXAMINE: a multicentre, randomised, double-blind trial. *Lancet*. 2015;385:2067–2076. doi: 10.1016/S0140-6736(14)62225-X.
38. McCormick LM, Hoole SP, White PA, Read PA, Axell RG, Clarke SJ, O'Sullivan M, West NE, Dutka DP. Pre-treatment with glucagon-like Peptide-1 protects against ischemic left ventricular dysfunction and stunning without a detected difference in myocardial substrate utilization. *JACC Cardiovasc Interv*. 2015;8:292–301. doi: 10.1016/j.jcin.2014.09.014.
39. Read PA, Khan FZ, Dutka DP. Cardioprotection against ischaemia induced by dobutamine stress using glucagon-like peptide-1 in patients with coronary artery disease. *Heart*. 2012;98:408–413. doi: 10.1136/hrt.2010.219345.
40. Read PA, Hoole SP, White PA, Khan FZ, O'Sullivan M, West NE, Dutka DP. A pilot study to assess whether glucagon-like peptide-1 protects the heart from ischemic dysfunction and attenuates stunning after coronary balloon occlusion in humans. *Circ Cardiovasc Interv*. 2011;4:266–272. doi: 10.1161/CIRCINTERVENTIONS.110.960476.
41. Nikolaidis LA, Mankad S, Sokos GG, Miske G, Shah A, Elahi D, Shannon RP. Effects of glucagon-like peptide-1 in patients with acute myocardial infarction and left ventricular dysfunction after successful reperfusion. *Circulation*. 2004;109:962–965. doi: 10.1161/01.CIR.0000120505.91348.58.
42. Lønborg J, Vejlstrup N, Kelbæk H, Bøtker HE, Kim WY, Mathiasen AB, Jørgensen E, Helqvist S, Saunamäki K, Clemmensen P, Holmvang L, Thuesen L, Krusell LR, Jensen JS, Køber L, Treiman M, Holst JJ, Engstrøm T. Exenatide reduces reperfusion injury in patients with ST-segment elevation myocardial infarction. *Eur Heart J*. 2012;33:1491–1499. doi: 10.1093/eurheartj/ehr309.
43. Woo JS, Kim W, Ha SJ, Kim JB, Kim SJ, Kim WS, Seon HJ, Kim KS. Cardioprotective effects of exenatide in patients with ST-segment-elevation myocardial infarction undergoing primary percutaneous coronary intervention: results of exenatide myocardial protection in revascularization study. *Arterioscler Thromb Vasc Biol*. 2013;33:2252–2260. doi: 10.1161/ATVBAHA.113.301586.
44. Chen WR, Hu SY, Chen YD, Zhang Y, Qian G, Wang J, Yang JJ, Wang ZF, Tian F, Ning QX. Effects of liraglutide on left ventricular function in patients with ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention. *Am Heart J*. 2015;170:845–854. doi: 10.1016/j.ahj.2015.07.014.
45. Chen WR, Shen XQ, Zhang Y, Chen YD, Hu SY, Qian G, Wang J, Yang JJ, Wang ZF, Tian F. Effects of liraglutide on left ventricular function in patients with non-ST-segment elevation myocardial infarction. *Endocrine*. 2016;52:516–526. doi: 10.1007/s12020-015-0798-0.
46. McCormick LM, Kydd AC, Read PA, Ring LS, Bond SJ, Hoole SP, Dutka DP. Chronic dipeptidyl peptidase-4 inhibition with sitagliptin is associated with sustained protection against ischemic left ventricular dysfunction in a pilot study of patients with type 2 diabetes mellitus and coronary artery disease. *Circ Cardiovasc Imaging*. 2014;7:274–281. doi: 10.1161/CIRCIMAGING.113.000785.
47. Read PA, Khan FZ, Heck PM, Hoole SP, Dutka DP. DPP-4 inhibition by sitagliptin improves the myocardial response to dobutamine stress and mitigates stunning in a pilot study of patients with coronary artery disease. *Circ Cardiovasc Imaging*. 2010;3:195–201. doi: 10.1161/CIRCIMAGING.109.899377.
48. Berkelaar M, Eekhoff EM, Simonis-Bik AM, Boomsma DI, Diamant M, Ijzerman RG, Dekker JM, 't Hart LM, de Geus EJ. Effects of induced hyperinsulinaemia with and without hyperglycaemia on measures of cardiac vagal control. *Diabetologia*. 2013;56:1436–1443. doi: 10.1007/s00125-013-2848-6.
49. Sun F, Wu S, Guo S, Yu K, Yang Z, Li L, Zhang Y, Quan X, Ji L, Zhan S. Impact of GLP-1 receptor agonists on blood pressure, heart rate and hypertension among patients with type 2 diabetes: a systematic review and network meta-analysis. *Diabetes Res Clin Pract*. 2015;110:26–37. doi: 10.1016/j.diabres.2015.07.015.

50. Robinson LE, Holt TA, Rees K, Randeva HS, O'Hare JP. Effects of exenatide and liraglutide on heart rate, blood pressure and body weight: systematic review and meta-analysis. *BMJ Open*. 2013;3.
51. Smits MM, Muskiet MH, Tonneijck L, Hoekstra T, Kramer MH, Diamant M, van Raalte DH. Exenatide acutely increases heart rate in parallel with augmented sympathetic nervous system activation in healthy overweight males. *Br J Clin Pharmacol*. 2016;81:613–620. doi: 10.1111/bcp.12843.
52. Mistry GC, Maes AL, Lasseter KC, Davies MJ, Gottesdiener KM, Wagner JA, Herman GA. Effect of sitagliptin, a dipeptidyl peptidase-4 inhibitor, on blood pressure in nondiabetic patients with mild to moderate hypertension. *J Clin Pharmacol*. 2008;48:592–598. doi: 10.1177/0091270008316885.
53. Aronis KN, Chamberland JP, Mantzoros CS. GLP-1 promotes angiogenesis in human endothelial cells in a dose-dependent manner, through the Akt, Src and PKC pathways. *Metabolism*. 2013;62:1279–1286. doi: 10.1016/j.metabol.2013.04.010.
54. Erdogdu O, Nathanson D, Sjöholm A, Nyström T, Zhang Q. Exendin-4 stimulates proliferation of human coronary artery endothelial cells through eNOS-, PKA- and PI3K/Akt-dependent pathways and requires GLP-1 receptor. *Mol Cell Endocrinol*. 2010;325:26–35. doi: 10.1016/j.mce.2010.04.022.
55. Ding L, Zhang J. Glucagon-like peptide-1 activates endothelial nitric oxide synthase in human umbilical vein endothelial cells. *Acta Pharmacol Sin*. 2012;33:75–81. doi: 10.1038/aps.2011.149.
56. Basu A, Charkoudian N, Schrage W, Rizza RA, Basu R, Joyner MJ. Beneficial effects of GLP-1 on endothelial function in humans: dampening by glyburide but not by glimepiride. *Am J Physiol Endocrinol Metab*. 2007;293:E1289–E1295. doi: 10.1152/ajpendo.00373.2007.
57. Nyström T, Gutniak MK, Zhang Q, Zhang F, Holst JJ, Ahrén B, Sjöholm A. Effects of glucagon-like peptide-1 on endothelial function in type 2 diabetes patients with stable coronary artery disease. *Am J Physiol Endocrinol Metab*. 2004;287:E1209–E1215. doi: 10.1152/ajpendo.00237.2004.
58. Wei R, Ma S, Wang C, Ke J, Yang J, Li W, Liu Y, Hou W, Feng X, Wang G, Hong T. Exenatide exerts direct protective effects on endothelial cells through the AMPK/Akt/eNOS pathway in a GLP-1 receptor-dependent manner. *Am J Physiol Endocrinol Metab*. 2016;310:E947–E957. doi: 10.1152/ajpendo.00400.2015.
59. Koska J, Sands M, Burciu C, D'Souza KM, Raravikar K, Liu J, Truran S, Franco DA, Schwartz EA, Schwenke DC, D'Alessio D, Migrino RQ, Reaven PD. Exenatide protects against glucose- and lipid-induced endothelial dysfunction: evidence for direct vasodilation effect of GLP-1 receptor agonists in humans. *Diabetes*. 2015;64:2624–2635. doi: 10.2337/db14-0976.
60. Schisano B, Harte AL, Lois K, Saravanan P, Al-Daghri N, Al-Attas O, Knudsen LB, McTernan PG, Ceriello A, Tripathi G. GLP-1 analogue, liraglutide protects human umbilical vein endothelial cells against high glucose induced endoplasmic reticulum stress. *Regul Pept*. 2012;174:46–52. doi: 10.1016/j.regpep.2011.11.008.
61. Shiraki A, Oyama J, Komoda H, Asaka M, Komatsu A, Sakuma M, Kodama K, Sakamoto Y, Kotooka N, Hirase T, Node K. The glucagon-like peptide 1 analog liraglutide reduces TNF-α-induced oxidative stress and inflammation in endothelial cells. *Atherosclerosis*. 2012;221:375–382. doi: 10.1016/j.atherosclerosis.2011.12.039.
62. Dai Y, Mehta JL, Chen M. Glucagon-like peptide-1 receptor agonist liraglutide inhibits endothelin-1 in endothelial cell by repressing nuclear factor-kappa B activation. *Cardiovasc Drugs Ther*. 2013;27:371–380. doi: 10.1007/s10557-013-6463-z.
63. Nandy D, Johnson C, Basu R, Joyner M, Brett J, Svendsen CB, Basu A. The effect of liraglutide on endothelial function in patients with type 2 diabetes. *Diab Vasc Dis Res*. 2014;11:419–430. doi: 10.1177/1479164114547358.
64. Matsubara J, Sugiyama S, Akiyama E, Iwashita S, Kurokawa H, Ohba K, Maeda H, Fujisue K, Yamamoto E, Kaikita K, Hokimoto S, Jinnouchi H, Ogawa H. Dipeptidyl peptidase-4 inhibitor, sitagliptin, improves endothelial dysfunction in association with its anti-inflammatory effects in patients with coronary artery disease and uncontrolled diabetes. *Circ J*. 2013;77:1337–1344.
65. Kubota Y, Miyamoto M, Takagi G, Ikeda T, Kirinoki-Ichikawa S, Tanaka K, Mizuno K. The dipeptidyl peptidase-4 inhibitor sitagliptin improves vascular endothelial function in type 2 diabetes. *J Korean Med Sci*. 2012;27:1364–1370. doi: 10.3346/jkms.2012.27.11.1364.
66. Hage C, Brismar K, Lundman P, Norhammar A, Rydén L, Mellbin L. The DPP-4 inhibitor sitagliptin and endothelial function in patients with acute coronary syndromes and newly detected glucose perturbations: a report from the BEGAMI study. *Diab Vasc Dis Res*. 2014;11:290–293. doi: 10.1177/1479164114533355.
67. Ayaori M, Iwakami N, Uto-Kondo H, Sato H, Sasaki M, Komatsu T, Iizuka M, Takiguchi S, Yakushiji E, Nakaya K, Yogo M, Ogura M, Takase B, Murakami T, Ikewaki K. Dipeptidyl peptidase-4 inhibitors attenuate endothelial function as evaluated by flow-mediated vasodilatation in type 2 diabetic patients. *J Am Heart Assoc*. 2013;2:e003277. doi: 10.1161/JAHA.112.003277.
68. Rizzo M, Chandalia M, Patti AM, Di Bartolo V, Rizvi AA, Montalto G, Abate N. Liraglutide decreases carotid intima-media thickness in patients with type 2 diabetes: 8-month prospective pilot study. *Cardiovasc Diabetol*. 2014;13:49. doi: 10.1186/1475-2840-13-49.
69. Ishikawa S, Shimano M, Watarai M, Koyasu M, Uchikawa T, Ishii H, Inden Y, Takemoto K, Murohara T. Impact of sitagliptin on carotid intima-media thickness in patients with coronary artery disease and impaired glucose tolerance or mild diabetes mellitus. *Am J Cardiol*. 2014;114:384–388. doi: 10.1016/j.amjcard.2014.04.050.
70. Mita T, Katakami N, Yoshii H, Onuma T, Kaneto H, Osonoi T, Shiraiwa T, Kosugi K, Umayahara Y, Yamamoto T, Yokoyama H, Kuribayashi N, Jinnouchi H, Gosho M, Shimomura I, Watada H; Collaborators on the Study of Preventive Effects of Alogliptin on Diabetic Atherosclerosis (SPEAD-A) Trial. Alogliptin, a dipeptidyl peptidase 4 inhibitor, prevents the progression of carotid atherosclerosis in patients with type 2 diabetes: the Study of Preventive Effects of Alogliptin on Diabetic Atherosclerosis (SPEAD-A). *Diabetes Care*. 2016;39:139–148. doi: 10.2337/dc15-0781.
71. Asmar A, Simonsen L, Asmar M, Madsbad S, Holst JJ, Frandsen E, Moro C, Jonassen T, Bülow J. Renal extraction and acute effects of glucagon-like peptide-1 on central and renal hemodynamics in healthy men. *Am J Physiol Endocrinol Metab*. 2015;308:E641–E649. doi: 10.1152/ajpendo.00429.2014.
72. Karstoft K, Mortensen SP, Knudsen SH, Solomon TP. Direct effect of incretin hormones on glucose and glycerol metabolism and hemodynamics. *Am J Physiol Endocrinol Metab*. 2015;308:E426–E433. doi: 10.1152/ajpendo.00520.2014.
73. Skov J, Holst JJ, Gøtze JP, Frøkiær J, Christiansen JS. Glucagon-like peptide-1: effect on pro-atrial natriuretic peptide in healthy males. *Endocr Connect*. 2014;3:11–16. doi: 10.1530/EC-13-0087.
74. von Scholten BJ, Lajer M, Goetze JP, Persson F, Rossing P. Time course and mechanisms of the anti-hypertensive and renal effects of liraglutide treatment. *Diabet Med*. 2015;32:343–352. doi: 10.1111/dme.12594.
75. Skov J, Pedersen M, Holst JJ, Madsen B, Goetze JP, Rittig S, Jonassen T, Frøkiaer J, Dejgaard A, Christiansen JS. Short-term effects of liraglutide on kidney function and vasoactive hormones in type 2 diabetes: a randomized clinical trial. *Diabetes Obes Metab*. 2016;18:581–589. doi: 10.1111/dom.12651.
76. Lovshin JA, Barnie A, DeAlmeida A, Logan A, Zinman B, Drucker DJ. Liraglutide promotes natriuresis but does not increase circulating levels of atrial natriuretic peptide in hypertensive subjects with type 2 diabetes. *Diabetes Care*. 2015;38:132–139. doi: 10.2337/dc14-1958.
77. Li CJ, Yu Q, Yu P, Yu TL, Zhang QM, Lu S, Yu DM. Changes in liraglutide-induced body composition are related to modifications in plasma cardiac natriuretic peptides levels in obese type 2 diabetic patients. *Cardiovasc Diabetol*. 2014;13:36. doi: 10.1186/1475-2840-13-36.
78. Muskiet MH, Tonneijck L, Smits MM, Kramer MH, Diamant M, Joles JA, van Raalte DH. Acute renal haemodynamic effects of glucagon-like peptide-1 receptor agonist exenatide in healthy overweight men. *Diabetes Obes Metab*. 2016;18:178–185. doi: 10.1111/dom.12601.
79. Tonneijck L, Smits MM, Muskiet MH, Hoekstra T, Kramer MH, Danser AH, Diamant M, Joles JA, van Raalte DH. Acute renal effects of the GLP-1 receptor agonist exenatide in overweight type 2 diabetes patients: a randomised, double-blind, placebo-controlled trial. *Diabetologia*. 2016;59:1412–1421. doi: 10.1007/s00125-016-3938-z.
80. Pfeffer MA, Claggett B, Diaz R, Dickstein K, Gerstein HC, Køber LV, Lawson FC, Ping L, Wei X, Lewis EF, Maggioni AP, McMurray JJ, Probstfield JL, Riddle MC, Solomon SD, Tardif JC; ELIXA Investigators. Lixisenatide in patients with type 2 diabetes and acute coronary syndrome. *N Engl J Med*. 2015;373:2247–2257. doi: 10.1056/NEJMoa1509225.
81. Groop PH, Cooper ME, Perkovic V, Emser A, Woerle HJ, von Eynatten M. Linagliptin lowers albuminuria on top of recommended standard treatment in patients with type 2 diabetes and renal dysfunction. *Diabetes Care*. 2013;36:3460–3468. doi: 10.2337/dc13-0323.
82. Nauck MA, Kleine N, Orskov C, Holst JJ, Willms B, Creutzfeldt W. Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7-36 amide) in type 2 (non-insulin-dependent) diabetic patients. *Diabetologia*. 1993;36:741–744.

83. Meier JJ, Gethmann A, Götze O, Gallwitz B, Holst JJ, Schmidt WE, Nauck MA. Glucagon-like peptide 1 abolishes the postprandial rise in triglyceride concentrations and lowers levels of non-esterified fatty acids in humans. *Diabetologia*. 2006;49:452–458. doi: 10.1007/s00125-005-0126-y.
84. Schwartz EA, Koska J, Mullin MP, Syoufi I, Schwenke DC, Reaven PD. Exenatide suppresses postprandial elevations in lipids and lipoproteins in individuals with impaired glucose tolerance and recent onset type 2 diabetes mellitus. *Atherosclerosis*. 2010;212:217–222. doi: 10.1016/j.atherosclerosis.2010.05.028.
85. Hermansen K, Bækdal TA, Düring M, Pietraszek A, Mortensen LS, Jørgensen H, Flint A. Liraglutide suppresses postprandial triglyceride and apolipoprotein B48 elevations after a fat-rich meal in patients with type 2 diabetes: a randomized, double-blind, placebo-controlled, cross-over trial. *Diabetes Obes Metab*. 2013;15:1040–1048. doi: 10.1111/dom.12133.
86. Tremblay AJ, Lamarche B, Deacon CF, Weisnagel SJ, Couture P. Effect of sitagliptin therapy on postprandial lipoprotein levels in patients with type 2 diabetes. *Diabetes Obes Metab*. 2011;13:366–373. doi: 10.1111/j.1463-1326.2011.01362.x.
87. Matikainen N, Mänttäri S, Schweizer A, Ulvestad A, Mills D, Dunning BE, Foley JE, Taskinen MR. Vildagliptin therapy reduces postprandial intestinal triglyceride-rich lipoprotein particles in patients with type 2 diabetes. *Diabetologia*. 2006;49:2049–2057. doi: 10.1007/s00125-006-0340-2.
88. Noda Y, Miyoshi T, Oe H, Ohno Y, Nakamura K, Toh N, Kohno K, Morita H, Kusano K, Ito H. Alogliptin ameliorates postprandial lipemia and postprandial endothelial dysfunction in non-diabetic subjects: a preliminary report. *Cardiovasc Diabetol*. 2013;12:8. doi: 10.1186/1475-2840-12-8.
89. Sharma S, Mells JE, Fu PP, Saxena NK, Anania FA. GLP-1 analogs reduce hepatocyte steatosis and improve survival by enhancing the unfolded protein response and promoting macroautophagy. *PLoS One*. 2011;6:e25269. doi: 10.1371/journal.pone.0025269.
90. Shao N, Kuang HY, Hao M, Gao XY, Lin WJ, Zou W. Benefits of exenatide on obesity and non-alcoholic fatty liver disease with elevated liver enzymes in patients with type 2 diabetes. *Diabetes Metab Res Rev*. 2014;30:521–529. doi: 10.1002/dmrr.2561.
91. Armstrong MJ, Gaunt P, Aithal GP, Barton D, Hull D, Parker R, Hazlehurst JM, Guo K, Abouda G, Aldersley MA, Stocken D, Gough SC, Tomlinson JW, Brown RM, Hübscher SG, Newsome PN; LEAN trial team. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. *Lancet*. 2016;387:679–690. doi: 10.1016/S0140-6736(15)00803-X.
92. Macauley M, Hollingsworth KG, Smith FE, Thelwall PE, Al-Mrabeh A, Schweizer A, Foley JE, Taylor R. Effect of vildagliptin on hepatic steatosis. *J Clin Endocrinol Metab*. 2015;100:1578–1585. doi: 10.1210/jc.2014-3794.
93. Cui J, Philo L, Nguyen P, Hofflich H, Hernandez C, Bettencourt R, Richards L, Salotti J, Bhatt A, Hooker J, Haufe W, Hooker C, Brenner DA, Sirlin CB, Loomba R. Sitagliptin vs. placebo for non-alcoholic fatty liver disease: a randomized controlled trial. *J Hepatol*. 2016;65:369–376. doi: 10.1016/j.jhep.2016.04.021.
94. Wang R, Lu L, Guo Y, Lin F, Chen H, Chen W, Chen M. Effect of glucagon-like peptide-1 on high-glucose-induced oxidative stress and cell apoptosis in human endothelial cells and its underlying mechanism. *J Cardiovasc Pharmacol*. 2015;66:135–140. doi: 10.1097/FJC.0000000000000255.
95. Chaudhuri A, Ghanim H, Vora M, Sia CL, Korzeniewski K, Dhindsa S, Makdissi A, Dandona P. Exenatide exerts a potent antiinflammatory effect. *J Clin Endocrinol Metab*. 2012;97:198–207. doi: 10.1210/jc.2011-1508.
96. Bułdak Ł, Łabuzek K, Bułdak RJ, Machnik G, Bołdys A, Okopień B. Exenatide (a GLP-1 agonist) improves the antioxidative potential of *in vitro* cultured human monocytes/macrophages. *Naunyn Schmiedebergs Arch Pharmacol*. 2015;388:905–919. doi: 10.1007/s00210-015-1124-3.
97. Makdissi A, Ghanim H, Vora M, Green K, Abuaysheh S, Chaudhuri A, Dhindsa S, Dandona P. Sitagliptin exerts an antinflammatory action. *J Clin Endocrinol Metab*. 2012;97:3333–3341. doi: 10.1210/jc.2012-1544.
98. Daousi C, Pinkney JH, Cleator J, Wilding JP, Ranganath LR. Acute peripheral administration of synthetic human GLP-1 (7-36 amide) decreases circulating IL-6 in obese patients with type 2 diabetes mellitus: a potential role for GLP-1 in modulation of the diabetic pro-inflammatory state? *Regul Pept*. 2013;183:54–61. doi: 10.1016/j.regpep.2013.03.004.
99. Hogan AE, Gaoatswe G, Lynch L, Corrigan MA, Woods C, O'Connell J, O'Shea D. Glucagon-like peptide 1 analogue therapy directly modulates innate immune-mediated inflammation in individuals with type 2 diabetes mellitus. *Diabetologia*. 2014;57:781–784. doi: 10.1007/s00125-013-3145-0.
100. Tremblay AJ, Lamarche B, Deacon CF, Weisnagel SJ, Couture P. Effects of sitagliptin therapy on markers of low-grade inflammation and cell adhesion molecules in patients with type 2 diabetes. *Metabolism*. 2014;63:1141–1148. doi: 10.1016/j.metabol.2014.06.004.
101. Bunck MC, Diamant M, Eliasson B, Cornér A, Shaginian RM, Heine RJ, Taskinen MR, Yki-Järvinen H, Smith U. Exenatide affects circulating cardiovascular risk biomarkers independently of changes in body composition. *Diabetes Care*. 2010;33:1734–1737. doi: 10.2337/dc09-2361.
102. Plutzky J, Garber A, Falahati A, Toft AD, Poulter NR. Reductions in lipids and CV risk markers in patients with type 2 diabetes treated with liraglutide: a meta-analysis. *Can J Diabetes*. 2009;33:209–210. doi: 10.1016/S1499-2671(09)33072-5.
103. Sahebkar A, Ponzo V, Bo S. Effect of dipeptidyl peptidase-4 inhibitors on plasma adiponectin: a systematic review and meta-analysis of randomized controlled trials. *Curr Med Chem*. 2016;23:1356–1369.
104. Cameron-Vendrig A, Reheman A, Siraj MA, Xu XR, Wang Y, Lei X, Afroze T, Shikatani E, El-Mounayri O, Noyan H, Weissleder R, Ni H, Husain M. Glucagon-like peptide 1 receptor activation attenuates platelet aggregation and thrombosis. *Diabetes*. 2016;65:1714–1723. doi: 10.2337/db15-1141.
105. Scheen AJ. Cardiovascular effects of dipeptidyl peptidase-4 inhibitors: from risk factors to clinical outcomes. *Postgrad Med*. 2013;125:7–20. doi: 10.3810/pgm.2013.05.2659.
106. Fadini GP, Boscaro E, Albiero M, Menegazzo L, Frison V, de Kreutzenberg S, Agostini C, Tiengo A, Avogaro A. The oral dipeptidyl peptidase-4 inhibitor sitagliptin increases circulating endothelial progenitor cells in patients with type 2 diabetes: possible role of stromal-derived factor-1alpha. *Diabetes Care*. 2010;33:1607–1609. doi: 10.2337/dc10-0187.
107. Gross L, Theiss HD, Grabmaier U, Adrion C, Mansmann U, Sohn HY, Hoffmann E, Steinbeck G, Franz WM, Brenner C. Combined therapy with sitagliptin plus granulocyte-colony stimulating factor in patients with acute myocardial infarction: long-term results of the SITAGRAMI trial. *Int J Cardiol*. 2016;215:441–445. doi: 10.1016/j.ijcard.2016.04.134.
108. Müssig K, Oncü A, Lindauer P, Heininger A, Aebert H, Unertl K, Ziemer G, Häring HU, Holst JJ, Gallwitz B. Effects of intravenous glucagon-like peptide-1 on glucose control and hemodynamics after coronary artery bypass surgery in patients with type 2 diabetes. *Am J Cardiol*. 2008;102:646–647. doi: 10.1016/j.amjcard.2008.06.029.
109. Chen WR, Chen YD, Tian F, Yang N, Cheng LQ, Hu SY, Wang J, Yang JJ, Wang SF, Gu XF. Effects of liraglutide on reperfusion injury in patients with ST-segment-elevation myocardial infarction. *Circ Cardiovasc Imaging*. 2016;9:XX–XX. doi: 10.1161/CIRCIMAGING.116.005146.
110. Meier JJ, Rosenstock J, Hincelin-Méry A, Roy-Duval C, Delfolie A, Coester HV, Menge BA, Forst T, Kapitza C. Contrasting effects of lixisenatide and liraglutide on postprandial glycemic control, gastric emptying, and safety parameters in patients with type 2 diabetes on optimized insulin glargine with or without metformin: a randomized, open-label trial. *Diabetes Care*. 2015;38:1263–1273. doi: 10.2337/dc14-1984.
111. Kim M, Platt MJ, Shibasaki T, Quaggin SE, Backx PH, Seino S, Simpson JA, Drucker DJ. GLP-1 receptor activation and Epac2 link atrial natriuretic peptide secretion to control of blood pressure. *Nat Med*. 2013;19:567–575. doi: 10.1038/nm.3128.
112. Chaudhuri A, Ghanim H, Makdissi A, Green K, Abuaysheh S, Batra M, D Kuhadiya N, Dandona P. Exenatide induces an increase in vasodilatory and a decrease in vasoconstrictive mediators. *Diabetes Obes Metab*. 2017;19:729–733. doi: 10.1111/dom.12835.
113. Skov J, Dejgaard A, Frøkiær J, Holst JJ, Jonassen T, Rittig S, Christiansen JS. Glucagon-like peptide-1 (GLP-1): effect on kidney hemodynamics and renin-angiotensin-aldosterone system in healthy men. *J Clin Endocrinol Metab*. 2013;98:E664–E671. doi: 10.1210/jc.2012-3855.
114. Mosenzon O, Leibowitz G, Bhatt DL, Cahn A, Hirshberg B, Wei C, Im K, Rozenberg A, Yanuv I, Stahre C, Ray KK, Iqbal N, Braunwald E, Scirica BM, Raz I. Effect of saxagliptin on renal outcomes in the SAVOR-TIMI 53 trial. *Diabetes Care*. 2017;40:69–76. doi: 10.2337/dc16-0621.
115. Groop PH, Cooper ME, Perkovic V, Sharma K, Schernthaner G, Haneda M, Hocher B, Gordat M, Cescutti J, Woerle HJ, von Eynatten M. Dipeptidyl peptidase-4 inhibition with linagliptin and effects on hyperglycaemia and albuminuria in patients with type 2 diabetes and renal dysfunction: rationale and design of the MARLINA-T2D™ trial. *Diab Vasc Dis Res*. 2015;12:455–462. doi: 10.1177/1479164115579002.
116. Xiao C, Dash S, Morgantini C, Patterson BW, Lewis GF. Sitagliptin, a DPP-4 inhibitor, acutely inhibits intestinal lipoprotein particle secretion in healthy humans. *Diabetes*. 2014;63:2394–2401. doi: 10.2337/db13-1654.

117. De Man FH, Cabezas MC, Van Barlingen HH, Erkelens DW, de Bruin TW. Triglyceride-rich lipoproteins in non-insulin-dependent diabetes mellitus: post-prandial metabolism and relation to premature atherosclerosis. *Eur J Clin Invest*. 1996;26:89–108.
118. Guleria A, Duseja A, Kalra N, Das A, Dhiman R, Chawla Y, Bhansali A. Patients with non-alcoholic fatty liver disease (NAFLD) have an increased risk of atherosclerosis and cardiovascular disease. *Trop Gastroenterol*. 2013;34:74–82.
119. Steven S, Jurk K, Kopp M, Kroller-Schon S, Mikhed Y, Schwierczek K, Roohani S, Kashani F, Oelze M, Klein T, Tokalov S, Danckwardt S, Strand S, Wenzel P, Munzel T, Daiber A. Glucagon-like peptide-1 receptor signalling reduces microvascular thrombosis, nitrosative and oxidative stress, systemic inflammation and platelet activation in endotoxaemic mice. *Br J Pharmacol*. 2016; 174: 1620–1632.
120. Rowley JW, Oler AJ, Tolley ND, Hunter BN, Low EN, Nix DA, Yost CC, Zimmerman GA, Weyrich AS. Genome-wide RNA-seq analysis of human and mouse platelet transcriptomes. *Blood*. 2011;118:e101–e111. doi: 10.1182/blood-2011-03-339705.
121. Balestrieri ML, Rizzo MR, Barbieri M, Paolisso P, D'Onofrio N, Giovane A, Siniscalchi M, Minicucci F, Sardu C, D'Andrea D, Mauro C, Ferraraccio F, Servillo L, Chirico F, Caiazzo P, Paolisso G, Marfella R. Sirtuin 6 expression and inflammatory activity in diabetic atherosclerotic plaques: effects of incretin treatment. *Diabetes*. 2015;64:1395–1406. doi: 10.2337/db14-1149.
122. Moretto TJ, Milton DR, Ridge TD, Macconell LA, Okerson T, Wolka AM, Brodows RG. Efficacy and tolerability of exenatide monotherapy over 24 weeks in antidiabetic drug-naive patients with type 2 diabetes: a randomized, double-blind, placebo-controlled, parallel-group study. *Clin Ther*. 2008;30:1448–1460. doi: 10.1016/j.clinthera.2008.08.006.
123. Simó R, Guerci B, Schernthaner G, Gallwitz B, Rosas-Guzmàn J, Dotta F, Festa A, Zhou M, Kiljański J. Long-term changes in cardiovascular risk markers during administration of exenatide twice daily or glimepiride: results from the European exenatide study. *Cardiovasc Diabetol*. 2015;14:116. doi: 10.1186/s12933-015-0279-z.
124. Rosenstock J, Raccah D, Korányi L, Maffei L, Boka G, Miossec P, Gerich JE. Efficacy and safety of lixisenatide once daily versus exenatide twice daily in type 2 diabetes inadequately controlled on metformin: a 24-week, randomized, open-label, active-controlled study (GetGoal-X). *Diabetes Care*. 2013;36:2945–2951. doi: 10.2337/dc12-2709.
125. Rosenstock J, Hanefeld M, Shamanna P, Min KW, Boka G, Miossec P, Zhou T, Muehlen-Bartmer I, Ratner RE. Beneficial effects of once-daily lixisenatide on overall and postprandial glycemic levels without significant excess of hypoglycemia in type 2 diabetes inadequately controlled on a sulfonylurea with or without metformin (GetGoal-S). *J Diabetes Complications*. 2014;28:386–392. doi: 10.1016/j.jdiacomp.2014.01.012.
126. Nauck M, Frid A, Hermansen K, Shah NS, Tankova T, Mitha IH, Zdravkovic M, Düring M, Matthews DR; LEAD-2 Study Group. Efficacy and safety comparison of liraglutide, glimepiride, and placebo, all in combination with metformin, in type 2 diabetes: the LEAD (liraglutide effect and action in diabetes)-2 study. *Diabetes Care*. 2009;32:84–90. doi: 10.2337/dc08-1355.
127. Buse JB, Nauck M, Forst T, Sheu WH, Shenouda SK, Heilmann CR, Hoogwerf BJ, Gao A, Boardman MK, Fineman M, Porter L, Schernthaner G. Exenatide once weekly versus liraglutide once daily in patients with type 2 diabetes (DURATION-6): a randomised, open-label study. *Lancet*. 2013;381:117–124. doi: 10.1016/S0140-6736(12)61267-7.
128. Dungan KM, Povedano ST, Forst T, González JG, Atisso C, Sealls W, Fahrbach JL. Once-weekly dulaglutide versus once-daily liraglutide in metformin-treated patients with type 2 diabetes (AWARD-6): a randomised, open-label, phase 3, non-inferiority trial. *Lancet*. 2014;384:1349–1357. doi: 10.1016/S0140-6736(14)60976-4.
129. Drucker DJ, Buse JB, Taylor K, Kendall DM, Trautmann M, Zhuang D, Porter L; DURATION-1 Study Group. Exenatide once weekly versus twice daily for the treatment of type 2 diabetes: a randomised, open-label, non-inferiority study. *Lancet*. 2008;372:1240–1250. doi: 10.1016/S0140-6736(08)61206-4.
130. Diamant M, Van Gaal L, Stranks S, Northrup J, Cao D, Taylor K, Trautmann M. Once weekly exenatide compared with insulin glargine titrated to target in patients with type 2 diabetes (DURATION-3): an open-label randomised trial. *Lancet*. 2010;375:2234–2243. doi: 10.1016/S0140-6736(10)60406-0.
131. Nauck M, Weinstock RS, Umpierrez GE, Guerci B, Skrivanek Z, Milicevic Z. Efficacy and safety of dulaglutide versus sitagliptin after 52 weeks in type 2 diabetes in a randomized controlled trial (AWARD-5). *Diabetes Care*. 2014;37:2149–2158. doi: 10.2337/dc13-2761.
132. Pratley RE, Nauck MA, Barnett AH, Feinglos MN, Ovalle F, Harman-Boehm I, Ye J, Scott R, Johnson S, Stewart M, Rosenstock J; HARMONY 7 Study Group. Once-weekly albiglutide versus once-daily liraglutide in patients with type 2 diabetes inadequately controlled on oral drugs (HARMONY 7): a randomised, open-label, multicentre, non-inferiority phase 3 study. *Lancet Diabetes Endocrinol*. 2014;2:289–297. doi: 10.1016/S2213-8587(13)70214-6.
133. Nauck MA, Stewart MW, Perkins C, Jones-Leone A, Yang F, Perry C, Reinhardt RR, Rendell M. Efficacy and safety of once-weekly GLP-1 receptor agonist albiglutide (HARMONY 2): 52 week primary endpoint results from a randomised, placebo-controlled trial in patients with type 2 diabetes mellitus inadequately controlled with diet and exercise. *Diabetologia*. 2016;59:266–274. doi: 10.1007/s00125-015-3795-1.
134. Nauck MA, Petrie JR, Sesti G, Mannucci E, Courrèges JP, Lindegaard ML, Jensen CB, Atkin SL; Study 1821 Investigators. A phase 2, randomized, dose-finding study of the novel once-weekly human GLP-1 analog, semaglutide, compared with placebo and open-label liraglutide in patients with type 2 diabetes. *Diabetes Care*. 2016;39:231–241. doi: 10.2337/dc15-0165.
135. Hanefeld M, Herman GA, Wu M, Mickel C, Sanchez M, Stein PP; Sitagliptin Study 014 Investigators. Once-daily sitagliptin, a dipeptidyl peptidase-4 inhibitor, for the treatment of patients with type 2 diabetes. *Curr Med Res Opin*. 2007;23:1329–1339. doi: 10.1185/030079907X188152.
136. Pratley RE, Nauck M, Bailey T, Montanya E, Cuddihy R, Filetti S, Thomsen AB, Søndergaard RE, Davies M; 1860-LIRA-DPP-4 Study Group. Liraglutide versus sitagliptin for patients with type 2 diabetes who did not have adequate glycaemic control with metformin: a 26-week, randomised, parallel-group, open-label trial. *Lancet*. 2010;375:1447–1456. doi: 10.1016/S0140-6736(10)60307-8.
137. Charbonnel B, Karasik A, Liu J, Wu M, Meininger G; Sitagliptin Study 020 Group. Efficacy and safety of the dipeptidyl peptidase-4 inhibitor sitagliptin added to ongoing metformin therapy in patients with type 2 diabetes inadequately controlled with metformin alone. *Diabetes Care*. 2006;29:2638–2643. doi: 10.2337/dc06-0706.
138. Dejager S, Razac S, Foley JE, Schweizer A. Vildagliptin in drug-naïve patients with type 2 diabetes: a 24-week, double-blind, randomized, placebo-controlled, multiple-dose study. *Horm Metab Res*. 2007;39:218–223. doi: 10.1055/s-2007-970422.
139. Evans M, Schweizer A, Foley JE. Blood pressure and fasting lipid changes after 24 weeks' treatment with vildagliptin: a pooled analysis in >2,000 previously drug-naïve patients with type 2 diabetes mellitus. *Vasc Health Risk Manag*. 2016;12:337–340. doi: 10.2147/VHRM.S112148.
140. Jadzinsky M, Pfützner A, Paz-Pacheco E, Xu Z, Allen E, Chen R; CV181-039 Investigators. Saxagliptin given in combination with metformin as initial therapy improves glycaemic control in patients with type 2 diabetes compared with either monotherapy: a randomized controlled trial. *Diabetes Obes Metab*. 2009;11:611–622. doi: 10.1111/j.1463-1326.2009.01056.x.
141. Rosenstock J, Aguilar-Salinas C, Klein E, Nepal S, List J, Chen R; CV181-011 Study Investigators. Effect of saxagliptin monotherapy in treatment-naïve patients with type 2 diabetes. *Curr Med Res Opin*. 2009;25:2401–2411. doi: 10.1185/03007990903178735.
142. Zinman B, Ahrén B, Neubacher D, Patel S, Woerle HJ, Johansen OE. Efficacy and cardiovascular safety of linagliptin as an add-on to insulin in type 2 diabetes: a pooled comprehensive post hoc analysis. *Can J Diabetes*. 2016;40:50–57. doi: 10.1016/j.jcjd.2015.06.010.
143. DeFronzo RA, Fleck PR, Wilson CA, Mekki Q; Alogliptin Study 010 Group. Efficacy and safety of the dipeptidyl peptidase-4 inhibitor alogliptin in patients with type 2 diabetes and inadequate glycemic control: a randomized, double-blind, placebo-controlled study. *Diabetes Care*. 2008;31:2315–2317. doi: 10.2337/dc08-1035.
144. Abd El Aziz MS, Kahle M, Meier JJ, Nauck MA. A meta-analysis comparing clinical effects of short- or long-acting GLP-1 receptor agonists versus insulin treatment from head-to-head studies in type 2 diabetic patients. *Diabetes Obes Metab*. 2017; 19: 216–227.
145. Zoungas S, Patel A, Chalmers J, de Galan BE, Li Q, Billot L, Woodward M, Ninomiya T, Neal B, MacMahon S, Grobbee DE, Kengne AP, Marre M, Heller S; ADVANCE Collaborative Group. Severe hypoglycemia and risks of vascular events and death. *N Engl J Med*. 2010;363:1410–1418. doi: 10.1056/NEJMoa1003795.
146. Monami M, Dicembrini I, Marchionni N, Rotella CM, Mannucci E. Effects of glucagon-like peptide-1 receptor agonists on body weight: a meta-analysis. *Exp Diabetes Res*. 2012;2012:672658. doi: 10.1155/2012/672658.
147. Nissen SE, Wolski K, Topol EJ. Effect of muraglitazar on death and major adverse cardiovascular events in patients with type 2 diabetes mellitus. *JAMA*. 2005;294:2581–2586. doi: 10.1001/jama.294.20.joc50147.

148. Nissen SE, Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *N Engl J Med*. 2007;356:2457–2471. doi: 10.1056/NEJMoa072761.
149. Scirica BM, Bhatt DL, Braunwald E, Steg PG, Davidson J, Hirshberg B, Ohman P, Price DL, Chen R, Udell J, Raz I. The design and rationale of the saxagliptin assessment of vascular outcomes recorded in patients with diabetes mellitus-thrombolysis in myocardial infarction (SAVOR-TIMI) 53 study. *Am Heart J*. 2011;162:818.e6–825.e6. doi: 10.1016/j.ahj.2011.08.006.
150. White WB, Bakris GL, Bergenstal RM, Cannon CP, Cushman WC, Fleck P, Heller S, Mehta C, Nissen SE, Perez A, Wilson C, Zannad F. EXamination of cArdiovascular outcoMes with alogliptIN versus standard of carE in patients with type 2 diabetes mellitus and acute coronary syndrome (EXAMINE): a cardiovascular safety study of the dipeptidyl peptidase 4 inhibitor alogliptin in patients with type 2 diabetes with acute coronary syndrome. *Am Heart J*. 2011;162:620.e1–626.e1. doi: 10.1016/j.ahj.2011.08.004.
151. Green JB, Bethel MA, Paul SK, Ring A, Kaufman KD, Shapiro DR, Califf RM, Holman RR. Rationale, design, and organization of a randomized, controlled Trial Evaluating Cardiovascular Outcomes with Sitagliptin (TECOS) in patients with type 2 diabetes and established cardiovascular disease. *Am Heart J*. 2013;166:983.e7–989.e7. doi: 10.1016/j.ahj.2013.09.003.
152. Bentley-Lewis R, Aguilar D, Riddle MC, Claggett B, Diaz R, Dickstein K, Gerstein HC, Johnston P, Køber LV, Lawson F, Lewis EF, Maggioni AP, McMurray JJ, Ping L, Probstfield JL, Solomon SD, Tardif JC, Wu Y, Pfeffer MA; ELIXA Investigators. Rationale, design, and baseline characteristics in Evaluation of LIXisenatide in Acute Coronary Syndrome, a long-term cardiovascular end point trial of lixisenatide versus placebo. *Am Heart J*. 2015;169:631.e7–638.e7. doi: 10.1016/j.ahj.2015.02.002.
153. Marso SP, Poulter NR, Nissen SE, Nauck MA, Zinman B, Daniels GH, Pocock S, Steinberg WM, Bergenstal RM, Mann JF, Ravn LS, Frandsen KB, Moses AC, Buse JB. Design of the liraglutide effect and action in diabetes: evaluation of cardiovascular outcome results (LEADER) trial. *Am Heart J*. 2013;166:823.e5–830.e5. doi: 10.1016/j.ahj.2013.07.012.
154. Engel SS, Golm GT, Shapiro D, Davies MJ, Kaufman KD, Goldstein BJ. Cardiovascular safety of sitagliptin in patients with type 2 diabetes mellitus: a pooled analysis. *Cardiovasc Diabetol*. 2013;12:3. doi: 10.1186/1475-2840-12-3.
155. McInnes G, Evans M, Del Prato S, Stumvoll M, Schweizer A, Lukashevich V, Shao Q, Kothny W. Cardiovascular and heart failure safety profile of vildagliptin: a meta-analysis of 17 000 patients. *Diabetes Obes Metab*. 2015;17:1085–1092. doi: 10.1111/dom.12548.
156. Frederich R, Alexander JH, Fiedorek FT, Donovan M, Berglind N, Harris S, Chen R, Wolf R, Mahaffey KW. A systematic assessment of cardiovascular outcomes in the saxagliptin drug development program for type 2 diabetes. *Postgrad Med*. 2010;122:16–27. doi: 10.3810/pgm.2010.05.2138.
157. White WB, Pratley R, Fleck P, Munsaka M, Hisada M, Wilson C, Menon V. Cardiovascular safety of the dipetidyl peptidase-4 inhibitor alogliptin in type 2 diabetes mellitus. *Diabetes Obes Metab*. 2013;15:668–673. doi: 10.1111/dom.12093.
158. Rosenstock J, Marx N, Neubacher D, Seck T, Patel S, Woerle HJ, Johansen OE. Cardiovascular safety of linagliptin in type 2 diabetes: a comprehensive patient-level pooled analysis of prospectively adjudicated cardiovascular events. *Cardiovasc Diabetol*. 2015;14:57. doi: 10.1186/s12933-015-0215-2.
159. Ratner R, Han J, Nicewarner D, Yushmanova I, Hoogwerf BJ, Shen L. Cardiovascular safety of exenatide BID: an integrated analysis from controlled clinical trials in participants with type 2 diabetes. *Cardiovasc Diabetol*. 2011;10:22. doi: 10.1186/1475-2840-10-22.
160. Marso SP, Lindsey JB, Stolker JM, House JA, Martinez Ravn G, Kennedy KF, Jensen TM, Buse JB. Cardiovascular safety of liraglutide assessed in a patient-level pooled analysis of phase 2: 3 liraglutide clinical development studies. *Diab Vasc Dis Res*. 2011;8:237–240. doi: 10.1177/1479164111408937.
161. Ferdinand KC, Botros FT, Atisso CM, Sager PT. Cardiovascular safety for once-weekly dulaglutide in type 2 diabetes: a pre-specified meta-analysis of prospectively adjudicated cardiovascular events. *Cardiovasc Diabetol*. 2016;15:38. doi: 10.1186/s12933-016-0355-z.
162. Fisher M, Petrie MC, Ambery PD, Donaldson J, Ye J, McMurray JJ. Cardiovascular safety of albiglutide in the Harmony programme: a meta-analysis. *Lancet Diabetes Endocrinol*. 2015;3:697–703. doi: 10.1016/S2213-8587(15)00233-8.
163. McGuire DK, Van de Werf F, Armstrong PW, Standl E, Koglin J, Green JB, Bethel MA, Cornel JH, Lopes RD, Halvorsen S, Ambrosio G, Buse JB, Josse RG, Lachin JM, Pencina MJ, Garg J, Lokhnygina Y, Holman RR, Peterson ED; Trial Evaluating Cardiovascular Outcomes With Sitagliptin Study Group. Association between sitagliptin use and heart failure hospitalization and related outcomes in type 2 diabetes mellitus: secondary analysis of a randomized clinical trial. *JAMA Cardiol*. 2016;1:126–135.
164. Toh S, Hampp C, Reichman ME, Graham DJ, Balakrishnan S, Pucino F, Hamilton J, Lendle S, Iyer A, Rucker M, Pimentel M, Nathwani N, Griffin MR, Brown NJ, Fireman BH. Risk for hospitalized heart failure among new users of saxagliptin, sitagliptin, and other antihyperglycemic drugs: a retrospective cohort study. *Ann Intern Med*. 2016;164:705–714. doi: 10.7326/M15-2568.
165. Margulies KB, Anstrom KJ, Hernandez AF, Redfield MM, Shah MR, Braunwald E, Cappola TP; Heart Failure Clinical Research Network. GLP-1 agonist therapy for advanced heart failure with reduced ejection fraction: design and rationale for the functional impact of GLP-1 for heart failure treatment study. *Circ Heart Fail*. 2014;7:673–679. doi: 10.1161/CIRCHEARTFAILURE.114.000346.
166. Gerstein HC, Miller ME, Genuth S, Ismail-Beigi F, Buse JB, Goff DC Jr, Probstfield JL, Cushman WC, Ginsberg HN, Bigger JT, Grimm RH Jr, Byington RP, Rosenberg YD, Friedewald WT; for the Accord Study Group. Long-term effects of intensive glucose lowering on cardiovascular outcomes. *N Engl J Med*. 2011;364:818–828.
167. Turnbull FM, Abraira C, Anderson RJ, Byington RP, Chalmers JP, Duckworth WC, Evans GW, Gerstein HC, Holman RR, Moritz TE, Neal BC, Ninomiya T, Patel AA, Paul SK, Travert F, Woodward M. Intensive glucose control and macrovascular outcomes in type 2 diabetes. *Diabetologia*. 2009;52:2288–2298.
168. 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;359:1577–1589. doi: 10.1056/NEJMoa0806470.
169. Gaede P, Lund-Andersen H, Parving HH, Pedersen O. Effect of a multifactorial intervention on mortality in type 2 diabetes. *N Engl J Med*. 2008;358:580–591. doi: 10.1056/NEJMoa0706245.
170. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, Mattheus M, Devins T, Johansen OE, Woerle HJ, Broedl UC, Inzucchi SE, Investigators E-RO. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med*. 2015;373:2117–2128.

Cardiovascular Actions and Clinical Outcomes With Glucagon-Like Peptide-1 Receptor Agonists and Dipeptidyl Peptidase-4 Inhibitors

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Supplemental Material

Cardiovascular actions and clinical outcomes with GLP-1R agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors

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1. Supplemental Tables

Supplementary table 1: GLP-1 receptors in the cardiovascular system and effects of GLP-1, GLP-1 receptor agonists and DPP-4 inhibitors (animal studies)

Organ	Effect(s) on	Description of effects	Negative findings, commentary
Heart	GLP-1 receptors	• Canonical GLP-1 R protein (immunocytochemistry) present in the ventricle of rodents [1,2] and non-human primates [3] • *GLP-1r* mRNA transcripts not detectable or expressed at very low level in the (right and left) ventricles, but primarily are found in atria [4,5] • GLP-1R protein (immunocytochemistry) in porcine and dog coronary arteries[6,7]	• Some published results have to be judged with caution because of low sensitivity and specificity of GLP-1 R antisera [8,9] • Some effects seem to be mediated by GLP-1 metabolites (GLP-1 [9-36 amide] or GLP-1 [28-36 amide] [10] • The exact mechanism how GLP-1 metabolites exert biological effects are not known. A second GLP-1 receptor with different ligand specificity has been looked for, but has not been detected or characterized [11,12] • GLP-1 metabolites appear to successfully target cytoplasmic and mitochondrial pathways leading to a reduction in reactive oxygen species in hepatocytes, endothelial cells, and cardiomyocytes[11,13] • GLP-1 (28-36) activates soluble adenylate cyclase and cAMP generation in isolated cardiomyocytes *ex vivo*[14] • Studies employing native GLP-1 may be associated with activation of dual cardiovascular pathways mediated through the classical GLP-1R and non-classical cAMP-mediated pathways activated by truncated peptides converging on cardiomyocyte and vascular protection[11].
	Myocardial glucose uptake	• GLP-1 (7-36 amide) or (7-37): Increased myocardial glucose uptake in dogs with cardiomyopathies and congestive heart failure[15,16] • GLP-1 receptor agonists: Not studied • DPP-4 inhibitors: Not studied	• Effects mimicked by GLP-1 [9-36 amide], the metabolite generated by DPP-4 action (which itself does not activate the canonical GLP-1 receptor) [11,15]
	Left-ventricular function	• GLP-1 (7-36 amide) or (7-37): Increased left-ventricular contractility [15] • GLP-1 receptor agonists: Liraglutide reduced myocardial tissue fibrosis induced by angiotensin II in rats[17] • DPP-4 inhibitors: Linagliptin was without effect on myocardial tissue fibrosis induced by angiotensin II in rats[17]	• Effects mimicked by GLP-1 [9-36 amide], the metabolite generated by DPP-4 action (which itself does not activate the canonical GLP-1 receptor) [15] • Experimental or clinical heart failure is associated with increased plasma DPP-4 activity[2] • Genetic disruption of DPP-4 (Fisher rats) or DPP-4 inhibition (vildagliptin) improves diastolic function in rats[18]. Pharmacological inhibition of DPP-4 (sitagliptin) attenuates heart failure in pigs[19]
	Myocardial/coronary blood flow	• GLP-1 (7-36 amide) or (7-37): Intracoronary GLP-1 did not change coronary flow in dogs (but increased myocardial glucose uptake)[6]. • Intravenous GLP-1 did not change coronary flow in swine [7]	• Also see table 1 (mostly studied in human subjects) • Effect of exogenous GLP-1 attenuated in obese animals[7]
	Cardioprotection against ischemia/ myocardial stunning	• GLP-1 (7-36 amide) or (7-37):See supplementary Figure 1 • GLP-1 receptor agonists: See supplementary Figure 1 • DPP-4 inhibitors: See supplementary Figure 1	• Uniformly, GLP-1, GLP-1 RAs and DPP-4Is reduce the area of necrosis (size of ultimate ischemic damage) after experimentally induced acute myocardial infarction (occlusion/ligation of a main coronary vessel) in mice, rats, pigs or dogs [20-29]. • Where studied, effects were antagonized by the GLP-1 receptor antagonist exendin [9-39] [30,31], indicating a major involvement of stimulated GLP-1 receptors
	Protection from stroke	• DPP-4 inhibitor: Linagliptin protects from stroke in non-diabetic and diabetic mice [32]	• It is unclear whether and how these findings are related to changes in GLP-1 concentrations induced by DPP-4 inhibition. Other mediators may be involved
	Heart rate	• GLP-1 (7-36 amide) or (7-37): Heart rate rapidly increased[12] • GLP-1 receptor agonists: Heart rate rapidly increased[12]; Exenatide reduced parasympathetic tone[33] • DPP-4 inhibitors: No change[12]	• Central and peripheral catecholaminergic pathways activated by GLP-1 in rodents[34] • The reduction in parasympathetic tone by GLP-1 R stimulation was not confirmed in human studies[35] • Mice with selective inactivation of GLP-1R in atrial cardiomyocytes exhibit a reduction in heart rate[2]

Supplementary table 1 continued on the next page

Supplementary table 1 (continued from previous page)

Organ	Effect(s) on	Description of effects	Negative findings, commentary
Peripheral arteries	GLP-1 receptors	• *GLP-1 r* mRNA transcripts have been detected within blood vessels[11], specifically to vascular smooth muscle cells in rodent and primate arterioles[3].	• Expression of GLP-1 R (protein level) in intact arterial or venous blood vessels is less certain [11]
	Angiogenesis, endothelial cell proliferation	• See table 1 (mostly studied in endothelial cells of human origin)	• Effects on endothelial cell proliferation and angiogenesis appear likely
	Endothelium-derived vasodilation (NO production)	• GLP-1 (7-36 amide) or (7-37) and GLP-1 receptor agonists: No sufficient evidence of direct vasodilatory action as an explanation for blood pressure-reducing effects [12]	• The role of direct vasodilation as a result of stimulating GLP-1 Rs remains unclear
	Anti-atherosclerotic effects	• GLP-1 (7-36 amide) or (7-37): GLP-1 attenuated the development of atherosclerosis in genetically susceptible rodent strains ($ApoE^{-/-}$)[36]	• Glucose-dependent insulinotropic polypeptide (GIP) also effective; DPP-4-degraded metabolites (GLP-1 [9-36 amide] or GIP [3-42]) were ineffective in this system.[36]
		• GLP-1 receptor agonists: Exendin-4 attenuated the development of atherosclerosis in genetically susceptible rodent strains ($ApoE^{-/-}$)[37] Liraglutide was effective in younger $ApoE^{-/-}$ mice with a low atherosclerotic burden, but not in older mice with more progressed atherosclerosis[38]	• GLP-1 receptor agonist taspoglutide treatment was without effect on the development of atherosclerosis or plaque formation in older mice with more progressed atherosclerosis[8]
		• DPP-4 inhibitors: Alogliptin attenuated the development of atherosclerosis in genetically susceptible rodent strains ($Ldlr^{-/-}$) [39]	• It is not known whether the effects reflect direct actions on blood vessels and plaque formation, or reductions in postprandial lipemia, weight loss, decreased vascular inflammation and/or attenuation of macrophage infiltration, or other mechanisms yet to be identified
	Systolic	• GLP-1 (7-36 amide) or (7-37): Acute increase in pulse rate and blood pressure[34]	• GLP-1R expression in renal vasculature reduced in a rodent model of arterial hypertension [40]
		• GLP-1 receptor agonists: Reduction (independent from weight loss); role of vasodilation unclear[11,12]	
		• DPP-4 inhibitors:	
Blood pressure	Natriuretic peptides	• GLP-1 receptor agonists: Liraglutide increases ANP and induces natroiuresis[4]	• Human studies do not uniformly support ANP as the relevant mediator of blood-pressure-lowering effects of GLP-1R stimulation (see table 1)
		• DPP-4 inhibitors: Little consistent effects on blood pressure	
Renal function	GLP-1 receptors	• GLP-1 receptor found in porcine proximal tubular cells [41]	• Some evidence for GLP-1 R expression in the renal *macula densa* (involved in local renin production?)[3]
	Glomerular filtration	• GLP-1 receptor agonists: : Exendin prevented increase in creatinine clearance (hyperfiltration) and glomerular hypertrophy in streptozotocin-induced diabetes in rats (model of type 1 diabetes)[42]	• No long-term studies in animal models of type 2 diabetes available
		• DPP-4 inhibitors: No obvious effects described	
	Albumin excretion	• GLP-1 receptor agonists: Exendin ameliorated albuminuria, mesangial expansion in streptozotocin-induced diabetes in rats (model of type 1 diabetes) [42]	• No long-term studies in animal models of type 2 diabetes available
	Natriuretic effects	• GLP-1 receptor agonists: Exendin increased natriuresis in obese mice[43]; effects on Na^+/H^+ exchange in renal proximal tubular cells [44]	• Effect of GLP-1 receptor agonist exenatide was(effects absent in GLP-1 R knock-out mice), but effect of DPP-4 inhibitor alogliptin was not mediated by the GLP-1 receptor [43]
		• DPP-4 inhibitors: Alogliptin increased natriuresis in obese mice[43]	
Metabolic milieu	Postprandial lipid concentrations	• GLP-1 receptor agonists: Exendin-4 reduced chylomicron production reduced in hamsters and mice [45]	• Chylomicron production increased in GLP-1R *knock-out* mice and with the receptor antagonist exendin [9-39]; sitagliptin effects antagonized by exendin [9-39] [45]
		• DPP-4 inhibitors: Sitagliptin reduced chylomicron production reduced in hamsters and mice [45]	

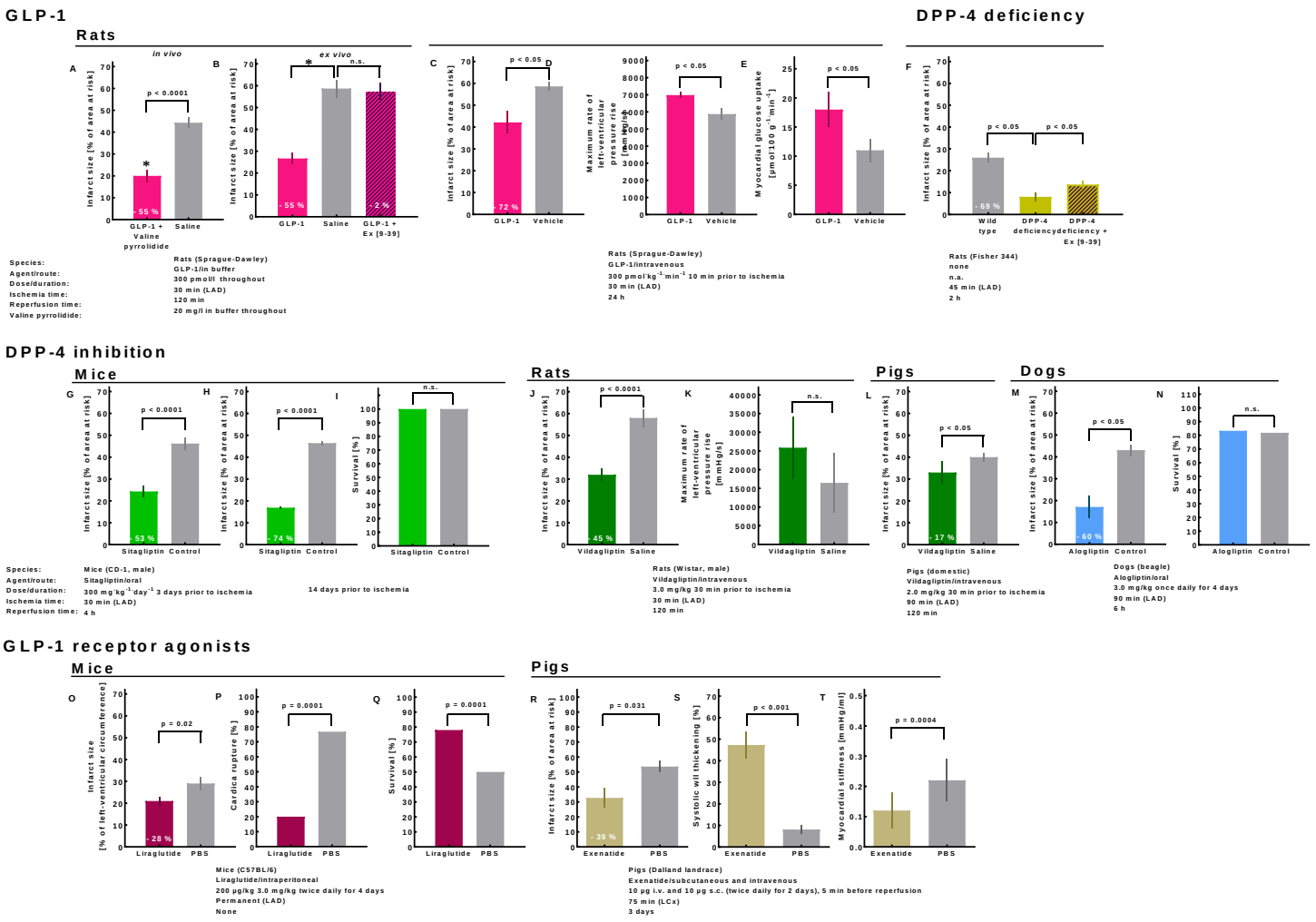
Supplementary table 1 continued on the next page

Supplementary table 1 (continued from previous page)

Organ	Effect(s) on	Description of effects	Negative findings, commentary
Liver	GLP-1 receptors Hepatic fat deposition (hepatic steatosis, NAFLD)	• Although binding sites for labeled GLP-1 have been described in hepatocytes, the expression of the canonical GLP-1 receptor in hepatocytes is unproven[46] • GLP-1 receptor agonists: Beneficial effects shown[48-50] • DPP-4 inhibitors: Mainly studied in human subjects (see table 1)	• GLP-1, nevertheless, has been shown to influence hepatic metabolism[47]. The mechanism may be indirect (through changes in hormonal levels or autonomic neural activity). • It is unclear as to whether improvements in glycemic control and weight loss contribute to beneficial effects on hepatic steatois/steatohepatitis
Inflammatory responses	GLP-1 receptors	• GLP-1 Rs are expressed at very low levels in circulating immune cells or lymphocytes isolated from thymus, spleen, or bone marrow (mice)[51] • Intestinal epithelial lymphocytes express higher levels of the canonical GLP-1 R[52]. • *GLP-1r* mRNA transcripts and immune-reactive GLP-1 R protein in macrophages [42] • Peritoneal and multiple tissue-derived macrophages without full-length *GLP-1r* mRNA[8] • Invariant natural killer T cells with questionable GLP-1R expression[53]	• GLP-1 R in intestinal epithelial lymphocytes coupled to classical cAMP-dependent anti-inflammatory signalling[52] • No obvious link between intestinal epithelial lymphocytes and cardiovascular functions of risks • Macrophage GLP-1 R not reproduced by Immunological Genome Project collaborative network (immgen.org)[12]
	Tissue inflammatory responses	• DPP-4 inhibitors: Sitagliptin reduced cardiac and systemic inflammation in rats with experimental heart failure[54] Alogliptin reduced arterial inflammation after femoral artery injury in $ldlr^{-/-}$ mice[39]	• Treatment of atherosclerosis-prone mice with DPP-4 inhibitors attenuates lesion formation, reduces plaque area, and decreases both systemic and vascular inflammation[2,55-57] • Young normoglycemic *Dpp4*$^{-/-}$ mice exhibit increased expression of proinflammatory genes in the heart[58] • Older diabetic high fat-fed mice with experimental heart failure exhibited increased cardiac inflammation following treatment with MK-0626[58] • The impact of reduced DPP-4 activity on cardiac inflammation may vary according to the experimental model under analysis.
Stem cell homing	SDF-1	• The CXCR4 antagonist AMD3100 attenuates beneficial effect attributed to DPP-4 inhibition in preclinical models of acute myocardial infarction[59] • Also studied in human subjects (see table 1)	• In the context of vascular and cardiac injury, DPP-4 controls stem cell homing, mediated through stabilization of SDF-1 (which mobilizes stem cells from bone marrow through a CXCR4-dependent mechanism.
Platelet function	Platelet aggregation	• GLP-1 receptor agonists: Mouse blood clot formation reduced by exenatide[60]. • DPP-4 inhibitors: Not studied in detail	• GLP-1R knock out increased blood clot formation[60] • This has mainly been studied in human subjects (see table 1)

ANP: Atrial natriuretic factor; ApoE: Apolipoprotein; cAMP: cyclic adenosine monophosphate; CXCR4: C-X-C chemokine receptor type 4, also known as fusin or stromal-derived factor-1 receptor; ldlr: Low-density lipoprotein (LDL) receptor; information presented in supplementary Table 1 is described in supplementary text (pages 6-8).

2. Supplemental Figures



Supplementary Figure 1. Effects of native GLP-1 ([7-36 amide]; A-E), DPP-4 inhibitors (sitagliptin, vildagliptin, alogliptin; G-N) and GLP-1 receptor agonists (liragutide, exenatide; O-T) in the size of the resulting necrotic area (relative to the area at risk) after standardized occlusion of a coronary artery in rats (A-F; J, K), mice (G-I, O-Q), pigs (L, R-T) and dogs (M, N). Essential experimental conditions (species studied; agent tested; route of administration; dose; duration of exposure; ischemia time; occluded coronary vessel; and reperfusion time) are provided in figure legends. In addition, parameters describing left-venticular function (D, E, K, S, T), the frequency of complications (P) and the proportion of animals surviving experimental acute myocardial infarction (I, N, Q) are depicted. The percentage reduction in infarct size (relative to the area at risk) is shown in white in the column representing treatment with GLP-1, DPP-4 inhibitor, or GLP-1 receptor agonist. LAD: Left anterior descendent (coronary artery); LCx: Left circumflex coronary artery; n.s.: not significant. Data have been taken from Bose et al. 2005 a[20] (A, B), Bose et al. 2005 b[61] (C-E), Ku et al. 2010 [24] (F), Ye te al.2010[25] (G-I), Chinda et al. 2014[27] (J,K), Chinda et al. 2013[26] (L), Ihara et al. 2015[28] (M, N), Noyan-Ashraf et al. 2009[21] (O-Q), and Timmers et al. 2009[22] (R-T). Details are described in the text (page 9).

3. Supplemental Text

GLP-1 Receptors in the Cardiovascular System (Including Central Nervous System Mechanisms Modifying Functions of the Cardiovascular System), see supplementary Table 1

GLP-1 Receptors in the Cardiovascular System. Mechanistic interpretation of the cardiovascular actions of GLP-1 requires ascertainment of the precise cells and tissues which express the canonical GLP-1R. Furthermore, some of the cardiovascular actions ascribed to native GLP-1, notably improvement of endothelial function, and reduction of oxidative stress, may reflect the activity of GLP-1 metabolites such as GLP-1 (9-36) and GLP-1 (28-36) which exert their actions independent of the known GLP-1 receptor [10,14]. Full length *Glp1r* mRNA transcripts have been detected in heart, blood vessels, cells of the immune system and in regions of the central nervous system that control autonomic function[12]. Some actions ascribed to GLP-1, such as reduction of natriuresis and attenuation of postprandial lipemia, likely arise through indirect GLP-1R-dependent mechanisms. Although GLP-1R-dependent activation of natriuresis and reduction of chylomicron secretion are mediated through the canonical GLP-1R [43,45], GLP-1 receptor mRNA transcripts are not detected within renal epithelium or intestinal enterocytes, respectively.

Several preclinical studies detected an immunoreactive GLP-1R protein by immunocytochemistry or western blot analysis within the ventricle of mice and rats}[2,62], however subsequent analyses have identified serious problems with the sensitivity and specificity of many GLP-1R antisera used in these experiments [8]. More recent studies have localized *Glp1r* mRNA transcripts predominantly to the atrium in rodents and primates. In contrast, *Glp1r* mRNA transcripts are expressed at very low levels or are not detectable in RNA isolated from the right and left cardiac ventricles in rodents[4,5]. The atrial expression of the GLP-1R protein was identified in non-human primate and human heart using a new highly specific monoclonal antibody, localizing an immunoreactive GLP-1R protein to cells within the sinoatrial node. Nevertheless, some studies have detected partial *GLP1R* mRNA transcripts by reverse transcription polymerase chain reaction techniques using RNA isolated from human ventricle, although GLP-1R agonists such as exenatide failed to augment contractility in the majority of isolated strips from human ventricle in the same experiments[63]. Nevertheless, RNASeq analyses have detected the presence of *GLP1R* mRNA transcripts in RNA from human left ventricle (http://www.gtexportal.org/home/gene/GLP1R). Hence, these findings raise the possibility that under some circumstances, translational control may dictate whether ventricular GLP-1R mRNA transcripts give rise to functional GLP-1R protein in the human heart.

Cardiovascular Effects of Stimulating GLP-1 Receptors with GLP-1 or GLP-1R Agonists – Animal studies

Heart Failure. Small short term exploratory studies with native GLP-1 have reported augmentation of ventricular function in animals with heart failure or ischemia-induced ventricular dysfunction[10]. Animal studies report increases in myocardial glucose uptake in dogs with experimental cardiomyopathy and congestive heart failure[15,16]. Critically, the therapeutic benefits of native GLP-1 were mimicked by independent administration of the truncated peptide, GLP-1 [9-36 amide], which does not meaningfully activate the canonical GLP-1R[15]. It seems likely that some of the cardiovascular benefits observed in preclinical and human studies with native GLP-1 reflect the bioactivity of one or more GLP-1 metabolites, such as GLP-1 [9-36] amide[11,12,15].

Blood vessels, endothelial function, atherosclerosis. *Glp1r* mRNA transcripts have been detected within blood vessels and infusion of native GLP-1 improves endothelial function in animals and human subjects, with and without diabetes (summarized in Pujadas & Drucker 2016[11]). Although the GLP-1R is expressed and functional in human endothelial cell lines, it remains uncertain whether the canonical GLP-1R is expressed in endothelial cells within intact blood vessels from specific arterial or venous beds. A functional GLP-1R has also been localized to vascular smooth muscle cells within rodent and primate renal arterioles[3]. Several preclinical studies report attenuation of experimental atherosclerosis in genetically sensitized rodent models, such as *apoE*-/- and *Ldlr*-/- mice treated with GLP-1R agonists[36,37]. In contrast, sustained GLP-1R agonism in older mice with more established

disease failed to attenuate the development or progression of atherosclerosis or plaque formation [8,38]. Whether the putative anti-atherogenic actions of GLP-1R agonists reflect direct actions on blood vessels, reduction of postprandial lipemia, weight loss, decreased vascular inflammation and /or attenuation of macrophage infiltration and plaque development, is difficult to ascertain.

Immune System and Inflammation. GLP-1 receptors are expressed at very low levels in circulating immune cells or in lymphocyte populations isolated from thymus, spleen, or bone marrow from male and female mice[51]. Moreover, whether peripheral circulating immune cells express a functional GLP-1R remains uncertain. In contrast, intestinal epithelial lymphocytes (IEL) express much higher levels of the canonical GLP-1R coupled to classical cyclic AMP-dependent anti-inflammatory signaling pathways[52]. However, there is no evidence linking signaling through the IEL GLP-1R to cardiovascular inflammation and the development of cardiovascular disease. Although some groups have reported expression of *Glp1r* mRNA transcripts and immunoreactive GLP-1R protein in macrophages[42,64], these findings were not reproduced in analyses of immune cell populations carried out by the Immunological Genome Project collaborative network (immgen.org)[12]. Similarly, analysis of multiple tissue and peritoneal mouse macrophage populations, analyzed in the basal state or following stimulation with thyoglycolate *in vivo* or phorbol 12-myristate 13-acetate *ex vivo* failed to detect expression of a full length *Glp1r* mRNA transcript in macrophage populations from wildtype, *Il10*$^{-/-}$ or *ApoE*$^{-/-}$ mice[8]. Although invariant natural killer T (iNKT) cells transduce some of the metabolic actions of GLP-1R agonists leading to weight loss in mice, whether iNKT cells express a functional GLP-1R remains uncertain[53]. Hence, the importance of macrophages and other immune cell types for anti-inflammatory actions of GLP-1R agonists requires more careful assessment.

Cardiac Function, Blood and Substrate Supply, Heart Rate. Infusion of native GLP-1 or GLP-1R agonists augments myocardial or coronary artery blood flow in some but not all[6,65] preclinical studies; whether these findings are secondary to increases in heart rate, or are mediated through hormonal, neural or vascular mechanisms independent of the classical GLP-1 receptor remains uncertain. Although GLP-1R expression has been detected by immunocytochemistry in coronary arteries from dogs and swine[6,7], a direct vasodilatory effect of GLP-1R agonists in the coronary vascular bed has not been described *in vivo*[6], or in isolated coronary artery preparations; hence the presence or absence of a functional GLP-1R in human coronary arteries remains uncertain[12]. Heart rate increases rapidly following administration of native GLP-1 or degradation-resistant GLP-1R agonists. In rodents, GLP-1R signaling activates central and peripheral catecholaminergic pathways[34], and reduces parasympathetic tone in some[33] but not all studies[35]. Notably, mice with selective inactivation of the GLP-1R in atrial cardiomyocytes exhibit a reduction in heart rate, underlining the physiological importance of basal cardiac GLP-1R signaling for rate control in rodents[2].

Blood Pressure. GLP-1R agonists also reduce blood pressure (BP) in animals with hypertension, independent of weight loss, through incompletely understood mechanisms[12]. Although native GLP-1 promotes vasorelaxation likely via GLP-1 [9-36 amide], there is insufficient evidence to support a direct vasodilatory action of GLP-1R agonists as a possible mechanism leading to lower blood pressure[11]. GLP-1R agonists increase secretion of atrial natriuretic factor (ANF) and indirectly promote natriuresis and vasodilation in hypertensive mice and rats, leading to lowering of blood pressure[4]. Nevertheless, human hypertensive subjects with T2D do not exhibit a significant increase in ANF following administration of GLP-1R agonists [66], hence the mechanisms linking GLP-1R signaling to reduction of blood pressure require additional scrutiny.

Platelet Aggregation. GLP-1R agonists also directly inhibit mouse platelet aggregation *ex vivo*, and reduces clot formation *in vivo* in rodents, findings mediated by the canonical GLP-1R[60]. Nevertheless, there is insufficient information to conclude whether this effect is direct, via a platelet GLP-1R, or indirect, and greater scrutiny of the mechanisms appears warranted.

Dipeptidyl Peptidase-4 (DPP-4) in the Cardiovascular System – Preclinical Studies

Heart failure. Experimental or clinical heart failure is associated with increased plasma DPP-4 activity[67] and genetic disruption or pharmacological inhibition of DPP-4 activity ameliorates multiple cardiovascular disorders in preclinical studies[68], including myocardial infarction[23,59], heart failure[18,19], aneurysm formation[69] and stroke[32]. Some of these therapeutic activities evident with DPP-4 inhibition

have been associated with increased levels of intact GLP-1, however most preclinical studies of DPP-4 inhibition invoke potential mechanisms through association, and comparatively few reports have used genetics or highly selective antagonists to probe the role of one or more cardioactive DPP4 substrates[68]. The beneficial effects of treatment (improved exercise capacity, enhanced mitochondrial biogenesis) with a DPP-4 inhibitor, MK-0626, in mice with myocardial infarction-associated heart failure were attenuated by co-administration of the GLP-1R antagonist exendin [9-39][30]. Similarly, treatment of mice with transaortic constriction (TAC)-induced heart failure for 4 weeks with alogliptin improved ventricular function, remodeling and decreased apoptosis, findings sensitive to co-administration of exendin [9-39][31]. Hence, some, but not all, of the benefits attributed to DPP-4 inhibition in preclinical studies of cardiovascular injury are secondary to enhanced GLP-1R signaling.

Stem cell homing. Compelling preclinical data supports a role for DPP-4 in the control of stem cell homing in the context of vascular and cardiac injury, mediated through stabilization of SDF-1, which in turn enhances bone marrow mobilization of stem cells through a CXCR4-dependent mechanism. Indeed, blockade of CXCR4 using the antagonist AMD3100 attenuates the beneficial effects attributed to DPP-4 inhibition in preclinical models of myocardial infarction[59]. Intriguingly, levels of circulating endothelial progenitor cells are reduced in subjects with type 2 diabetes, and increased following four weeks of treatment with a DPP-4 inhibitor[70]. Collectively these preclinical and clinical studies fostered the hypothesis that DPP-4 inhibitors might enhance stem cell homing to injured myocardium and attenuate myocardial injury in human subjects with acute coronary syndrome and myocardial infarction. The SITAgliptin plus GRanulocyte-colony-stimulating factor in patients suffering from Acute Myocardial Infarction (SITAGRAMI) study administered sitagliptin for 28 days and Granluocyte-Colony Stimulating Factor (G-CSF) for 5 days to human subjects after acute myocardial infarction, with a pre-specified primary endpoint of change in ventricular ejection fraction assessed by cardiac MRI (Table 1). After a mean follow-up period of 4.5 years, no difference in clinical outcomes or ejection fraction was detected in subjects assigned to placebo or sitagliptin-G-CSF combination therapy[71].

Inflammation. DPP-4 inhibition also produces robust attenuation of experimental inflammation in animal models. Reduction of cardiac and systemic inflammation has been observed in sitagliptin-treated rats with experimental heart failure[54], and two weeks of alogliptin administration reduced arterial inflammation following femoral artery injury in low-density lipoprotein receptor-deficient mice[39]. Treatment of atherosclerosis-prone mice with DPP-4 inhibitors attenuates lesion formation, reduces plaque area, and decreases both systemic and vascular inflammation[55-57,68]. Unexpectedly however, young normoglycemic *Dpp4*$^{-/-}$ mice exhibit increased expression of proinflammatory genes in the heart[58], and older diabetic high fat-fed mice with experimental heart failure exhibited increased cardiac inflammation following treatment with MK-0626[58]. Hence the impact of reduced DPP-4 activity on cardiac inflammation may vary according to the experimental model under analysis.

Other cardiovascular effects. Although neuropeptide Y is a physiological substrate for DPP-4, reduction of DPP-4 activity has little consistent effect on control of blood pressure in the majority of normotensive or hypertensive individuals. In contrast, reduction of DPP-4 activity acutely reduces postprandial lipemia through attenuation of Apo B-48-rich chylomicron secretion in normoglycemic or diabetic rodents [45]. These actions are mediated through potentiation of GLP-1R signaling in rodents and diminished by co-administration of exendin [9-39][45]. Hence, taken together, the available preclinical literature supports a mechanistic link between reduction of DPP-4 activity, and modification of cardiovascular risk factors as well as direct DPP-4substrate-mediated attenuation of cardiovascular injury in normoglycemic and diabetic animals.

Further details on effects of GLP-1, GLP-1RA and DPP-4I in experimental acute myocardial infarction (see supplemental Figure 1)

GLP-1 exerts cardioprotective properties when administered as a preconditioning mimetic or at the time of reperfusion[20]. In some studies, better left-ventricular performance (significantly higher maximum-developed left-ventricular pressure) was observed after exposure to either GLP-1 (supplementary Fig. 1 D)[20] or vildagliptin (supplementary Fig. 1 K)[27], consistent with a greater systolic wall thickening observed in pigs (supplementary Fig. 1 S)[22]. A significantly higher myocardial glucose uptake was described in rats exposed to GLP-1 prior to inducing ischemia (supplementary Fig. 1 E)[29] and this finding has been replicated in other studies[72]. A few studies reported endpoints encompassing i) complications and ii) survival after experimentally-induced myocardial infarction: Survival was not changed in two studies with DPP-4 inhibitors (sitagliptin in mice, supplementary Fig. 1 I[25]; alogliptin in dogs, supplementary Fig. 1 N[28]), but was improved in other studies involving DPP-4 inhibitor treatment with sitagliptin [23] or vildagliptin[73], and in mice treated with liraglutide for 4 days prior to a permanent ligation of the left anterior descending (LAD) coronary artery (supplementary Fig. 1 Q)[21]. Along the same lines, ventricular rupture was a common finding in placebo-treated animals, but was significantly reduced in animals treated with the GLP-1R agonist liraglutide (supplementary Fig. 1 P)[21]. Furthermore, Fisher rats with a genetic deficiency in DPP-4 exhibit higher concentrations of GLP-1 and exhibit reduced myocardial necrosis following induction of experimental ischemia (supplementary Fig. 1 F)[74]; exenatide [9-39] administration partially attenuated this cardioprotection[74]. Similar results were obtained by Sauvé et al. 2010, who demonstrated resistance to ischemic cardiac injury in *Dpp4*$^{-/-}$ mice, findings mimicked in wild-type diabetic mice treated with sitagliptin[23].

References

(quoted in supplementary Table 1, in the legend to supplementary Figure 1, and in text belonging to online supplementary material)

1. Ban K, Noyan-Ashraf MH, Hoefer J, Bolz SS, Drucker DJ, Husain M. Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. *Circulation*. 2008;117:2340-2350.
2. Ussher JR, Baggio LL, Campbell JE, Mulvihill EE, Kim M, Kabir MG, Cao X, Baranek BM, Stoffers DA, Seeley RJ, Drucker DJ. Inactivation of the cardiomyocyte glucagon-like peptide-1 receptor (GLP-1R) unmasks cardiomyocyte-independent GLP-1R-mediated cardioprotection. *Mol Metab*. 2014;3:507-517.
3. Pyke C, Heller RS, Kirk RK, Ørskov C, Reedtz-Runge S, Kaastrup P, Hvelplund A, Bardram L, Calatayud D, Knudsen LB. GLP-1 receptor localization in monkey and human tissue; Novel distribution revealed with extensively validated monoclonal antibody. *Endocrinology*. 2014;155:1280-1290.
4. Kim M, Platt M, Shibasaki T, Quaggin S, Backx PH, Seino S, Simpson J, Drucker DJ. GLP-1 receptor activation and Epac2 link atrial natriuretic peptide secretion to control of blood pressure. *Nat Med*. 2013;19:567-575.
5. Wohlfart P, Linz W, Hubschle T, Linz D, Huber J, Hess S, Crowther D, Werner U, Ruetten H. Cardioprotective effects of lixisenatide in rat myocardial ischemia-reperfusion injury studies. *J Translat Med*. 2013;11.
6. Moberly SP, Berwick ZC, Kohr M, Svendsen M, Mather KJ, Tune JD. Intracoronary glucagon-like peptide 1 preferentially augments glucose uptake in ischemic myocardium independent of changes in coronary flow. *Exp Biol Med (Maywood)*. 2012;237:334-342.
7. Moberly SP, Mather KJ, Berwick ZC, Owen MK, Goodwill AG, Casalini ED, Hutchins GD, Green MA, Ng Y, Considine RV, Perry KM, Chisholm RL, Tune JD. Impaired cardiometabolic responses to glucagon-like peptide 1 in obesity and type 2 diabetes mellitus. *Basic Res Cardiol*. 2013;108:365.
8. Panjwani N, Mulvihill EE, Longuet C, Yusta B, Campbell JE, Brown TJ, Streutker C, Holland D, Cao X, Baggio LL, Drucker DJ. GLP-1 receptor activation indirectly reduces hepatic lipid

accumulation but does not attenuate development of atherosclerosis in diabetic male ApoE-/- mice. *Endocrinology*. 2013;154:127-139.
9. Drucker DJ. Deciphering metabolic messages from the gut drives therapeutic innovation: the 2014 Banting Lecture. *Diabetes*. 2015;64:317-326.
10. Abu-Hamdah R, Rabiee A, Meneilly GS, Shannon RP, Andersen DK, Elahi D. Clinical review: The extrapancreatic effects of glucagon-like peptide-1 and related peptides. *J Clin Endocrinol Metab*. 2009;94:1843-1852.
11. Pujadas G, Drucker DJ. Vascular biology of glucagon receptor superfamily peptides: complexity, controversy, and clinical relevance. *Endocr Rev*. 2016:er20161078.
12. Drucker DJ. The cardiovascular biology of glucagon-like peptide-1. *Cell Metab*. 2016;24:15-30.
13. Giacco F, Du X, Carratu A, Gerfen GJ, D'Apolito M, Giardino I, Rasola A, Marin O, Divakaruni AS, Murphy AN, Shah MS, Brownlee M. GLP-1 cleavage product reverses persistent ROS generation after transient hyperglycemia by disrupting an ROS-generating feedback loop. *Diabetes*. 2015;64:3273-3284.
14. Mundil D, Beca S, Cameron-Vendriug A, El-Mounayri O, Momen A, Backx PH, Husain M. GLP-1 [28-36] exerts direct cardioprotective effects, activating pro-survival kinases and soluble adenylyl cyclase (abstract 13657). *Circulation*. 2012;126 (suppl. 21):A 13657.
15. 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:H2401-2408.
16. Bhashyam S, Fields AV, Patterson B, Testani JM, Chen L, Shen YT, Shannon RP. Glucagon-like peptide-1 increases myocardial glucose uptake via p38alpha MAP kinase-mediated, nitric oxide-dependent mechanisms in conscious dogs with dilated cardiomyopathy. *Circ Heart Fail*. 2010;3:512-521.
17. Zhang LH, Pang XF, Bai F, Wang NP, Shah AI, McKallip RJ, Li XW, Wang X, Zhao ZQ. Preservation of glucagon-like peptide-1 level attenuates angiotensin II-induced tissue fibrosis by altering AT1/AT 2 receptor expression and angiotensin-converting enzyme 2 activity in rat heart. *Cardiovasc Drugs Ther*. 2015;29:243-255.
18. Shigeta T, Aoyama M, Bando YK, Monji A, Mitsui T, Takatsu M, Cheng XW, Okumura T, Hirashiki A, Nagata K, Murohara T. Dipeptidyl peptidase-4 modulates left ventricular dysfunction in chronic heart failure via angiogenesis-dependent and -independent actions. *Circulation*. 2012;126:1838-1851.
19. Gomez N, Touihri K, Matheeussen V, Mendes Da Costa A, Mahmoudabady M, Mathieu M, Baerts L, Peace A, Lybaert P, Scharpe S, De Meester I, Bartunek J, Vanderheyden M, Mc Entee K. Dipeptidyl peptidase IV inhibition improves cardiorenal function in overpacing-induced heart failure. *Europ J Heart Fail*. 2012;14:14-21.
20. Bose AK, Mocanu MM, Carr RD, Brand CL, Yellon DM. Glucagon-like peptide 1 can directly protect the heart against ischemia/reperfusion injury. *Diabetes*. 2005;54:146-151.
21. Noyan-Ashraf MH, Momen MA, Ban K, Sadi AM, Zhou YQ, Riazi AM, Baggio LL, Henkelman RM, Husain M, Drucker DJ. GLP-1R agonist liraglutide activates cytoprotective pathways and improves outcomes after experimental myocardial infarction in mice. *Diabetes*. 2009;58:975-983.
22. Timmers L, Henriques JP, de Kleijn DP, Devries JH, Kemperman H, Steendijk P, Verlaan CW, Kerver M, Piek JJ, Doevendans PA, Pasterkamp G, Hoefer IE. Exenatide reduces infarct size and improves cardiac function in a porcine model of ischemia and reperfusion injury. *J Am Coll Cardiol*. 2009;53:501-510.
23. Sauvé M, Ban K, Momen A, Zhou Y-Q, Henkelman RM, Husain M, Drucker DJ. Genetic deletion or pharmacological inhibition of dipeptidyl peptidase-4 improves cardiovascular outcomes following myocardial infarction in mice. *Diabetes*. 2010;59:1063-1073.
24. Ku HC, Chen WP, Su MJ. GLP-1 signaling preserves cardiac function in endotoxemic Fischer 344 and DPP4-deficient rats. *Naunyn Schmiedebergs Arch Pharmacol*. 2010;382:463-474.
25. Ye Y, Keyes KT, Zhang C, Perez-Polo JR, Lin Y, Birnbaum Y. The myocardial infarct size-limiting effect of sitagliptin is PKA-dependent, whereas the protective effect of pioglitazone is partially dependent on PKA. *Am J Physiol (Heart Circ Physiol)*. 2010;298:H 1454-1465.

26. Chinda K, Palee S, Surinkaew S, Phornphutkul M, Chattipakorn S, Chattipakorn N. Cardioprotective effect of dipeptidyl peptidase-4 inhibitor during ischemia-reperfusion injury. *Int J Cardiol*. 2013;167:451-457.
27. Chinda K, Sanit J, Chattipakorn S, Chattipakorn N. Dipeptidyl peptidase-4 inhibitor reduces infarct size and preserves cardiac function via mitochondrial protection in ischaemia-reperfusion rat heart. *Diab Vasc Dis Res*. 2014;11:75-83.
28. Ihara M, Asanuma H, Yamazaki S, Kato H, Asano Y, Shinozaki Y, Mori H, Minamino T, Asakura M, Sugimachi M, Mochizuki N, Kitakaze M. An interaction between glucagon-like peptide-1 and adenosine contributes to cardioprotection of a dipeptidyl peptidase 4 inhibitor from myocardial ischemia-reperfusion injury. *Am J Physiol (Heart Circ Physiol)*. 2015;308:H 1287-1297.
29. Aravindhan K, Bao W, Harpel MR, Willette RN, Lepore JJ, Jucker BM. Cardioprotection resulting from glucagon-like peptide-1 administration involves shifting metabolic substrate utilization to increase energy efficiency in the rat heart. *PLoS One*. 2015;10:e0130894.
30. Takada S, Masaki Y, Kinugawa S, Matsumoto J, Furihata T, Mizushima W, Kadoguchi T, Fukushima A, Homma T, Takahashi M, Harashima S, Matsushima S, Yokota T, Tanaka S, Okita K, Tsutsui H. Dipeptidyl peptidase-4 inhibitor improved exercise capacity and mitochondrial biogenesis in mice with heart failure via activation of GLP-1 receptor signaling. *Cardiovasc Res*. 2016.
31. Aoyama M, Kawase H, Bando YK, Monji A, Murohara T. Dipeptidyl Peptidase 4 Inhibition Alleviates Shortage of Circulating Glucagon-Like Peptide-1 in Heart Failure and Mitigates Myocardial Remodeling and Apoptosis via the Exchange Protein Directly Activated by Cyclic AMP 1/Ras-Related Protein 1 Axis. *Circ Heart Fail*. 2016;9:e002081.
32. Darsalia V, Ortsater H, Olverling A, Darlof E, Wolbert P, Nyström T, Klein T, Sjöholm A, Patrone C. The DPP-4 inhibitor linagliptin counteracts stroke in the normal and diabetic mouse brain: a comparison with glimepiride. *Diabetes*. 2013;62:1289-1296.
33. Griffioen KJ, Wan R, Okun E, Wang X, Lovett-Barr MR, Li Y, Mughal MR, Mendelowitz D, Mattson MP. GLP-1 receptor stimulation depresses heart rate variability and inhibits neurotransmission to cardiac vagal neurons. *Cardiovasc Res*. 2011;89:72-78.
34. Yamamoto H, Lee CE, Marcus JN, Williams TD, Overton JM, Lopez ME, Hollenberg AN, Baggio L, Saper CB, Drucker DJ, Elmquist JK. Glucagon-like peptide-1 receptor stimulation increases blood pressure and heart rate and activates autonomic regulatory neurons. *J Clin Invest*. 2002;110:43-52.
35. Bharucha AE, Charkoudian N, Andrews CN, Camilleri M, Sletten D, Zinsmeister AR, Low PA. Effects of glucagon-like peptide-1, yohimbine, and nitrergic modulation on sympathetic and parasympathetic activity in humans. *Am J Physiol (Regul Integr Comp Physiol)* 2008;295:R874-880.
36. Nagashima M, Watanabe T, Terasaki M, Tomoyasu M, Nohtomi K, Kim-Kaneyama J, Miyazaki A, Hirano T. Native incretins prevent the development of atherosclerotic lesions in apolipoprotein E knockout mice. *Diabetologia*. 2011;54:2649-2659.
37. Arakawa M, Mita T, Azuma K, Ebato C, Goto H, Nomiyama T, Fujitani Y, Hirose T, Kawamori R, Watada H. Inhibition of monocyte adhesion to endothelial cells and attenuation of atherosclerotic lesion by a glucagon-like peptide-1 receptor agonist, exendin-4. *Diabetes*. 2010;59:1030-1037.
38. Gaspari T, Welungoda I, Widdop RE, Simpson RW, Dear AE. The GLP-1 receptor agonist liraglutide inhibits progression of vascular disease via effects on atherogenesis, plaque stability and endothelial function in an ApoE(-/-) mouse model. *Diab Vasc Dis Res*. 2013;10:353-360.
39. Akita K, Isoda K, Shimada K, Daida H. Dipeptidyl-peptidase-4 inhibitor, alogliptin, attenuates arterial inflammation and neointimal formation after injury in low-density lipoprotein (LDL) receptor-deficient mice. *J Am Heart Assoc*. 2015;4:e001469.
40. Liu L, Liu J, Gao Y, Ng CF, Yu X, Dou D, Huang Y. Protein kinase Cbeta mediates downregulated expression of glucagon-like peptide-1 receptor in hypertensive rat renal arteries. *J Hypertens*. 2015;33:784-90; discussion 790.
41. Schlatter P, Beglinger C, Drewe J, Gutmann H. Glucagon-like peptide 1 receptor expression in primary porcine proximal tubular cells. *Regul Pept*. 2007;141:120-128.

42. Kodera R, Shikata K, Kataoka HU, Takatsuka T, Miyamoto S, Sasaki M, Kajitani N, Nishishita S, Sarai K, Hirota D, Sato C, Ogawa D, Makino H. Glucagon-like peptide-1 receptor agonist ameliorates renal injury through its anti-inflammatory action without lowering blood glucose level in a rat model of type 1 diabetes. *Diabetologia*. 2011;54:965-978.
43. Rieg T, Gerasimova M, Murray F, Masuda T, Tang T, Rose M, Drucker DJ, Vallon V. The natriuretic effect by exendin-4, but not the DPP-4 inhibitor alogliptin, is mediated via the GLP-1 receptor and preserved in obese type 2 diabetic mice. *Am J Physiol (Renal Physiol)*. 2012;303:F 963-971.
44. Carraro-Lacroix LR, Malnic G, Girardi AC. Regulation of Na+/H+ exchanger NHE3 by glucagon-like peptide 1 receptor agonist exendin-4 in renal proximal tubule cells. *Am J Physiol (Renal Physiol)*. 2009;297:F 1647-1655.
45. Hsieh J, Longuet C, Baker CL, Qin B, Federico LM, Drucker DJ, Adeli K. The glucagon-like peptide 1 receptor is essential for postprandial lipoprotein synthesis and secretion. *Diabetologia*. 2010;53:552-561.
46. Pedersen J, Holst JJ. Glucagon like-peptide 1 receptor and the liver. *Liver Int*. 2011;31:1243-1245.
47. Seghieri M, Rebelos E, Gastaldelli A, Astiarraga BD, Casolaro A, Barsotti E, Pocai A, Nauck M, Muscelli E, Ferrannini E. Direct effect of GLP-1 infusion on endogenous glucose production in humans. *Diabetologia*. 2013;56:156-161.
48. Gupta NA, Kolachala VL, Jiang R, Abramowsky C, Romero R, Fifadara N, Anania F, Knechtle S, Kirk A. The glucagon-like peptide-1 receptor agonist exendin 4 has a protective role in ischemic injury of lean and steatotic liver by inhibiting cell death and stimulating lipolysis. *Am J Pathol*. 2012;181:1693-1701.
49. Wang XC, Gusdon AM, Liu H, Qu S. Effects of glucagon-like peptide-1 receptor agonists on non-alcoholic fatty liver disease and inflammation. *World J Gastroenterol*. 2014;20:14821-14830.
50. Mells JE, Fu PP, Sharma S, Olson D, Cheng L, Handy JA, Saxena NK, Sorescu D, Anania FA. Glp-1 analog, liraglutide, ameliorates hepatic steatosis and cardiac hypertrophy in C57BL/6J mice fed a Western diet. *Am J Physiol (Gastrointest Liver Physiol)*. 2012;302:G 225-235.
51. Hadjiyanni I, Siminovitch KA, Danska JS, Drucker DJ. Glucagon-like peptide-1 receptor signalling selectively regulates murine lymphocyte proliferation and maintenance of peripheral regulatory T cells. *Diabetologia*. 2010;53:730-740.
52. Yusta B, Baggio LL, Koehler J, Holland D, Cao X, Pinnell LJ, Johnson-Henry KC, Yeung W, Surette MG, Bang KA, Sherman PM, Drucker DJ. GLP-1 receptor (GLP-1R) agonists modulate enteric immune responses through the intestinal intraepithelial lymphocyte (IEL) GLP-1R. *Diabetes*. 2015;64:2537-2349.
53. Lynch L, Hogan AE, Duquette D, Drucker DJ, O'SHea D, Brenner M. iNKT cells induce FGF21 for thermogenesis and are required for maximal weight loss in GLP-1 therapy. *Cell Metab*. 2016;24:510-519.
54. de Almeida Salles T, Zogbi C, de Lima TM, de Godoi Carneiro C, Garcez AT, Barbeiro HV, Antonio EL, Dos Santos L, da Costa Pereira A, Tucci PJ, de Paula Faria D, Soriano FG, Girardi AC. The contributions of dipeptidyl peptidase IV to inflammation in heart failure. *Am J Physiol (Heart Circ Physiol)*. 2016;310:H 1760-1772.
55. Shah Z, Kampfrath T, Deiuliis JA, Zhong J, Pineda C, Ying Z, Xu X, Lu B, Moffatt-Bruce S, Durairaj R, Sun Q, Mihai G, Maiseyeu A, Rajagopalan S. Long-term dipeptidyl-peptidase 4 inhibition reduces atherosclerosis and inflammation via effects on monocyte recruitment and chemotaxis. *Circulation*. 2011;124:2338-2349.
56. Vittone F, Liberman A, Vasic D, Ostertag R, Esser M, Walcher D, Ludwig A, Marx N, Burgmaier M. Sitagliptin reduces plaque macrophage content and stabilises arteriosclerotic lesions in Apoe (-/-) mice. *Diabetologia*. 2012;55:2267-75.
57. Mulvihill EE, Drucker DJ. Pharmacology, physiology and mechanisms of action of dipeptidyl peptidase-4 inhibitors. *Endocr Rev*. 2014;6:992-1019.

58. Mulvihill EE, Varin EM, Ussher JR, Campbell JE, Bang KW, Abdullah T, Baggio LL, Drucker DJ. Inhibition of dipeptidyl peptidase-4 impairs ventricular function and promotes cardiac fibrosis in high fat-Fed diabetic mice. *Diabetes*. 2016;65:742-754.
59. Theiss HD, Vallaster M, Rischpler C, Krieg L, Zaruba MM, Brunner S, Vanchev Y, Fischer R, Grobner M, Huber B, Wollenweber T, Assmann G, Mueller-Hoecker J, Hacker M, Franz WM. Dual stem cell therapy after myocardial infarction acts specifically by enhanced homing via the SDF-1/CXCR4 axis. *Stem Cell Res*. 2011;7:244-255.
60. Cameron-Vendrig A, Reheman A, Siraj MA, Xu XR, Wang Y, Lei X, Afroze T, Shikatani E, El-Mounayri O, Noyan H, Weissleder R, Ni H, Husain M. Glucagon-like peptide-1 receptor activation attenuates platelet aggregation and thrombosis. *Diabetes*. 2016;65:1714-1723.
61. Bose AK, Mocanu MM, Carr RD, Yellon DM. Glucagon like peptide-1 is protective against myocardial ischemia/reperfusion injury when given either as a preconditioning mimetic or at reperfusion in an isolated rat heart model. *Cardiovasc Drugs Ther*. 2005;19:9-11.
62. Ussher JR, Drucker DJ. Cardiovascular biology of the incretin system. *Endocr Rev*. 2012;33:187-215.
63. Wallner M, Kolesnik E, Ablasser K, Khafaga M, Wakula P, Ljubojevic S, Thon-Gutschi EM, Sourij H, Kapl M, Edmunds NJ, Kuzmiski JB, Griffith DA, Knez I, Pieske B, von Lewinski D. Exenatide exerts a PKA-dependent positive inotropic effect in human atrial myocardium: GLP-1R mediated effects in human myocardium. *J Mol Cell Cardiol*. 2015;89:365-75.
64. Liang CP, Han S, Li G, Tabas I, Tall AR. Impaired MEK signaling and SERCA expression promote ER stress and apoptosis in insulin-resistant macrophages and are reversed by exenatide treatment. *Diabetes*. 2012;61:2609-2620.
65. Gejl M, Sondergaard HM, Stecher C, Bibby BM, Moller N, Botker HE, Hansen SB, Gjedde A, Rungby J, Brock B. Exenatide alters myocardial glucose transport and uptake depending on insulin resistance and increases myocardial blood flow in patients with type 2 diabetes. *J Clin Endocrinol Metab*. 2012;97:E 1165-1169.
66. Lovshin JA, Barnie A, DeAlmeida A, Logan A, Zinman B, Drucker DJ. Liraglutide promotes natriuresis but does not increase circulating levels of atrial natriuretic peptide in hypertensive subjects with type 2 diabetes. *Diabetes Care*. 2015;38:132-139.
67. Dos Santos L, Salles TA, Arruda-Junior DF, Campos LC, Pereira AC, Barreto AL, Antonio EL, Mansur AJ, Tucci PJ, Krieger JE, Girardi AC. Circulating dipeptidyl peptidase IV activity correlates with cardiac dysfunction in human and experimental heart failure. *Circ Heart Fail*. 2013;6:1029-1038.
68. Ussher JR, Drucker DJ. Cardiovascular actions of incretin-based therapies. *Circulation Res*. 2014;114:1788-1803.
69. Bao W, Morimoto K, Hasegawa T, Sasaki N, Yamashita T, Hirata K, Okita Y, Okada K. Orally administered dipeptidyl peptidase-4 inhibitor (alogliptin) prevents abdominal aortic aneurysm formation through an antioxidant effect in rats. *J Vasc Surg*. 2014;59:1098-108.
70. Fadini GP, Boscaro E, Albiero M, Menegazzo L, Frison V, de Kreutzenberg S, Agostini C, Tiengo A, Avogaro A. The oral dipeptidyl peptidase-4 inhibitor sitagliptin increases circulating endothelial progenitor cells in patients with type 2 diabetes mellitus. Possible role of stromal derived factor-1{alpha}. *Diabetes Care*. 2010;33:1607-9.
71. Gross L, Theiss HD, Grabmaier U, Adrion C, Mansmann U, Sohn HY, Hoffmann E, Steinbeck G, Franz WM, Brenner C. Combined therapy with sitagliptin plus granulocyte-colony stimulating factor in patients with acute myocardial infarction - Long-term results of the SITAGRAMI trial. *Int J Cardiol*. 2016;215:441-445.
72. Zhao T, Parikh P, Bhashyam S, Bolukoglu H, Poornima I, Shen YT, Shannon RP. Direct effects of glucagon-like peptide-1 on myocardial contractility and glucose uptake in normal and postischemic isolated rat hearts. *J Pharmacol Exp Ther*. 2006;317:1106-1113.
73. Murase H, Kuno A, Miki T, Tanno M, Yano T, Kouzu H, Ishikawa S, Tobisawa T, Ogasawara M, Nishizawa K, Miura T. Inhibition of DPP-4 reduces acute mortality after myocardial infarction with restoration of autophagic response in type 2 diabetic rats. *Cardiovasc Diabetol*. 2015;14:103.
74. Ku HC, Chen WP, Su MJ. DPP4 deficiency preserves cardiac function via GLP-1 signaling in rats subjected to myocardial ischemia/reperfusion. *Naunyn Schmiedebergs Arch Pharmacol*. 2011;384:197-207.