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REVIEW ARTICLES

Effects of Incretin Mimetic Drugs on Diabetic Cardiovascular Functions

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SUMMARY

Incretin effect has a key role in glycemic homeostasis following meals. Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are responsible for the incretin effect; they are released from the small intestine after meals and stimulate insulin secretion from pancreatic β-cells, thereby causing significant reduction in blood sugar level. These peptides have quite short half-lives, as they are inactivated within minutes by the enzyme dipeptidyl peptidase (DPP-4). In type 2 diabetes mellitus (T2DM), GLP-1 levels decrease, and incretin effect is reduced. This worsens the diabetic state. Two different groups of drugs, GLP-1 receptor agonists and DPP-4 inhibitors, have been developed in order to benefit from the favorable effects of GLP-1 on glucose homeostasis, and they have been since used in treatment of T2DM. Both groups of drugs show favorable effects on cardiovascular dysfunctions, independent of their blood glucose-lowering effects, and this feature distinguishes them from the conventional anti-hyperglycemic agents. Preclinical and clinical studies have shown that both GLP-1 receptor activation and DPP-4 inhibition exert multifaceted cardioprotection on cardiovascular dysfunction. In addition to providing glycemic control, treatment with incretin mimetic drugs help to control body weight, significantly reduce the risk of hypoglycemia, lower blood pressure and improve cardiovascular functions. Furthermore, by increasing the number of pancreatic β-cells, they ameliorate the pancreatic injury caused by diabetes and enhance insulin response. Considering all these benefits together, incretin mimetic drugs have been proposed as first-line medications in combined therapy of diabetic patients, especially in the presence of cardiovascular complications.

Key Words: *Diabetes, Cardiovascular, Antidiabetic, Incretin, GLP-1, DPP-4.*

İnkretin Mimetik İlaçların Diabetik Kardiyovasküler Fonksiyonlar Üzerindeki Etkileri

ÖZET

İnkretin etki, yemek sonrası glisemik homeostazın sağlanmasında etkin rol oynayan bir mekanizmadır. İnkretin etkiden sorumlu Glukagon benzeri peptid-1 (GLP-1) ve Glukoz-bağımlı insülinotropik polipeptid (GIP), yemek sonrası ince bağırsaktan salgılanarak pankreasın β-hücrelerinden insülin sekresyonunu arttırırlar, böylece kan şekerini belirgin olarak düşürürler. Söz konusu peptidler dakikalar içerisinde dipeptidil peptidaz enzimi (DPP-4) tarafından inaktive edildiklerinden yarılanma ömürleri oldukça kısadır. T2DM'de GLP-1 seviyeleri düşer ve inkretin etki azalır. Bu durum diyabet tablosunu daha da kötüleştirir. GLP-1'in glukoz dengesi üzerindeki olumlu etkilerinden yararlanmak için iki farklı ilaç grubu geliştirilmiş ve Tip 2 diyabet tedavisinde kullanılmaya başlanmıştır; GLP-1 reseptör agonistleri ve DPP-4 inhibitörleri. Her iki grup da kan şekerini düşürücü etkilerinden bağımsız olarak kardiyovasküler disfonksiyonlar üzerinde olumlu etki göstermektedir ve bu yönüyle geleneksel antihiperglisemik ajanlardan ayrılmaktadır. Preklinik ve klinik çalışmalarda GLP-1 reseptör aktivasyonu ve DPP-4 inhibisyonunun kardiyovasküler disfonksiyon üzerinde çok yönlü kardiyoprotektif etki gösterdiği kanıtlanmıştır. İnkretin mimetik ilaçlar ile tedavide glisemik kontrolün yanı sıra vücut ağırlığı kontrol altındadır, hipoglisemi riski çok düşüktür, ayrıca, kan basıncı düşer ve kardiyovasküler fonksiyonlarda iyileşme görülür. Bunlara ek olarak, pankreastaki β-hücre sayısını arttırarak diyabetin yol açtığı pankreas harabiyetini iyileştirir ve insülin cevabını arttırır. Tüm bu etkiler bir arada değerlendirildiğinde, özellikle diyabete bağlı kardiyovasküler komplikasyon gelişen diyabetik hastalarda inkretin mimetik ilaçların kombine tedavide ilk sırada tercih edilecek ilaç gruplarından biri olduğu öne sürülmüştür.

Anahtar kelimeler: *Diyabet, Kardiyovasküler, Antidiyabetik, İnkretin, GLP-1, DPP-4.*

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INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is the most important risk factor for cardiovascular disease development. Poor glycemic control is directly associated with microvascular and macrovascular complications of the disease. Uncontrolled diabetes often results in ventricular dysfunction (independent of coronary artery disease and hypertension), myocardial apoptosis, hypertrophy, and in the long-term it leads to cardiomyopathy that is characterized with fibrosis and cardiac dysfunction (Chong, Clarke, & Levelt, 2017). At the initial phase, asymptomatic diastolic dysfunction develops due to disturbances in left ventricular tissue structure and calcium ion transport, oxidative stress and changes in cardiac metabolism. The disturbed left ventricular structure leads to impaired left ventricular passive tension, preventing active relaxation during diastole. This leads to left ventricular hypertrophy. During later stages of the diabetes, systolic dysfunction adds to the diastolic dysfunction. Left ventricular ejection fraction is depressed due to systolic dysfunction (Gelzinis, 2014). Mortality rate in diabetic patients with cardiovascular complications is 2-4 folds higher than diabetic patients without complications.

The most commonly used agent in T2DM treatment is metformin. The UK Prospective Diabetes Study (UKPS) examined the differences between anti-hyperglycemic agents and their effects on T2DM patients; although the study did not find a significant cardiovascular improvement in patients using metformin alone since early stage of the disease, the same patients were re-evaluated 10 years after completion of the study, and a significant reduction was observed in macrovascular complication development and mortality rates ("Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group," 1998; Holman, Paul, Bethel, Matthews, & Neil, 2008). This suggests that although metformin therapy initiated at the early stage of diabetes does not have cardiovascular efficacy in the short-term, it has a protective effect against cardiac complications by establishing a "metabolic memory". Despite these beneficial effects on the heart, acceptable glucose levels could be achieved in approximately 50% of patients who received metformin for 3 years, and this rate dropped to 25% in 9 year-treatment (Fisman & Tenenbaum, 2015; Jayawardene et al., 2014; Turner, Cull, Frighi, & Holman, 1999).

In short, metformin does not appear sufficient alone for lowering blood glucose during a life-long treatment. In the long-term, metformin has to be combined with another anti-hyperglycemic drug that does not have cardiovascular side effects. The first consideration in routine practice is the sulphonylureas. Although they seem safe, studies have shown increased mortality; and increased risk for lactic acidosis particularly in elderly patients and patients with liver failure, when sulphonylureas are combined with metformin (Rao, Kuhadiya, Reynolds, & Fonseca, 2008). Furthermore, while sulphonylureas exert insulinotropic effect by closing down the ATP-dependent potassium channels, they cause adverse effects on the heart. They impair Ca^{++} flow by partially inhibiting opening of the channels during myocardial ischemia, and thus, they eliminate the hyperpolarization, which normally protects the cell (Kottenberg et al., 2014). Glinides, which act through closing the ATP-dependent potassium channels, also have cardiovascular risks similar to sulphonylureas. Clinical studies on T2DM patients have shown that metformin treatment combined with glinides (particularly glipizide) caused increased cardiovascular mortality rates (Mogensen et al., 2015). Glitazones, on the other hand, cause fluid retention, and therefore lead to edema, stroke, congestive heart failure and mortality risk due to these reasons. This risk is further increased in combined treatment with sulphonylurea and insulin (Wagstaff & Goa, 2002). For this reason glitazones are contraindicated in the presence of liver failure and congestive heart failure (NHYA Class 1-4), and should be used with caution in patients aged 65 years or above (Graham et al., 2010; Lu et al., 2013).

In summary, anti-hyperglycemic agents that are frequently used in clinical practice have varying degrees of cardiovascular side effects. Furthermore, recent studies have shown that although an effective glycemic control can reduce microvascular adverse events, it is not sufficient for prevention from macrovascular adverse events (Waldrop, Zhong, Peters, & Rajagopalan, 2016). From this viewpoint, the primary purpose of the treatment should be to achieve a good glycemic control without triggering hypoglycemia or cardivascular complications, and to provide cardiovascular protection. In that case, new antidiabetic drug groups that will have cardioprotective effect while regulating blood glucose are necessary.

Incretin mimetics, which are considered a new therapeutic approach in diabetes, have the desired cardiac-beneficial profile, as they show protective effects on the cardiovascular system through direct

and indirect ways (Saraiva & Sposito, 2014). These group of drugs enhance insulin sensitivity and lower blood glucose without the risk of hypoglycemia, aid in losing weight, give feeling of satiety, and exert favorable effects on metabolic pathways that might pose cardiovascular risk.

Incretin effect

The fact that orally administered glucose causes a higher insulin response than intravenous glucose administration is explained by the "incretin effect". Incretins are members of the glucagon superfamily secreted from the intestines (Waldrop et al., 2016). The two of the incretins called glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are secreted within minutes after meals, and are responsible for 70% of the insulin release response as part of the incretin effect (Goud, Zhong, Peters, Brook, & Rajagopalan, 2016).

GLP-1 is 80% responsible for this effect. After meals, GLP-1 is synthesized from the L-cells present in jejunum and distal ileum, and it binds to G-protein coupled receptors in the endocrine pancreas. It stimulates cyclic adenosine monophosphate (cAMP) production through adenylate cyclase, and stimulates insulin release from the pancreatic β-cells. It inhibits glucagon release by desensitizing pancreatic α-cells, and also inhibits hepatic gluconeogenesis. It additionally stimulates gene expression in pancreatic β-cells by increasing membrane permeability through ion channels (Waldrop et al., 2016), and it increases the cell mass, and prolongs the life of these cells as well. Thus, it lowers blood glucose level and maintains homeostasis (Burgmaier, Heinrich, & Marx, 2013). Since this response is glucose-dependent, the risk of hypoglycemia is low.

In addition, it slows down gastric motility after meals, and reduces rapid blood glucose elevations and diminishes appetite. It induces feeling of satiety by acting on the hypothalamus (Little et al., 2006). This action is important for normalizing postprandial glucose escalations and maintaining homeostasis. Several studies have shown peripheral effects of GLP-1 with reduction of plasma concentrations of free fatty acids (Dailey & Moran, 2013).

The effects of incretins are short-lived. The enzyme dipeptidylpeptidase-4 (DPP-4) that is released from the endothelial cells degrade the free GLP-1 and GIP to the inactive forms GLP-1[9-36] and GIP[3-42] within 1-3 minutes. This is the reason why they have short plasma half-lives (4-7 min.).

In T2DM, the levels of GLP-1 and GIP decrease, and the incretin effect reduces. This worsens the pathological condition. Because GLP-1 plays more effective role than GIP in the incretin effect, two drug groups have been developed for treatment of diabetes, in order to enhance GLP-1 concentration in the gastrointestinal system. These groups are named as GLP-1 agonists and DPP-4 inhibitors. GLP-1 receptor agonists reversibly bind to the GLP-1 receptors and directly stimulate insulin release, whereas DPP-4 inhibitors prevent degradation of GLP-1 and prolong their duration of action (Burgmaier et al., 2013). Both groups of drugs reduce blood glucose and HbA1c (glycosylated hemoglobin) to acceptable levels. Two of their most important properties are; unlike sulphonylureas, the risk of hypoglycemia is very low, and unlike thiazolinediaones or sulphonylureas, they do not cause weight gain (Scheen, 2012).

Incretin-based therapies: GLP-1 receptor agonists and DPP-4 inhibitors

GLP-1 receptor agonists

GLP-1 receptor agonists are synthetic complete receptor agonists displaying physiological actions of the natural incretin. They bind to GLP-1 receptors with high affinity, increase glucose-dependent insulin secretion and inhibit glucagon release; they delay gastric emptying, and reduce appetite (Goud et al., 2016). They increase the number pancreatic β-cells, and they reduce apoptosis in these cells as well.

Table 1 shows GLP-1 receptor agonists that are designed for clinical use. The most commonly used analogs are exenatide and liraglutide. Compared with the endogenous GLP-1, agonists are more resistant to DPP-4 action. Therefore, their duration of action is longer than GLP-1.

The first GLP-1 agonist introduced to the market was exenatide. Exenatide lowers HbA1c level of T2DM patients with HbA1c levels above 7% by a unit value of 0.5-1.0%. Long-acting exenatide is more effective in glycemic control compared to short-acting exenatide (1.5-1.9% reduction in HbA1c). In addition to lowering HbA1c level, it causes 3-5 kg of weight loss, and improved lipid profile (total cholesterol, LDL, TAG) and hepatic aminotransferases.

Nausea and vomiting can occur in 30% of patients at the initiation of treatment. These side effects are reduced if the dose is increased gradually when treatment is initiated for the first time. Apart from the gastrointestinal side effects, subcutaneous administration is another factor reducing patient compliance. Long-acting GLP-1 receptor agonists that are administered once a week were developed with the purpose of increasing patient compliance.

Table 1: GLP-1 analogs

	Administration route	Dose interval	Dose amount	Advantages	Approval
Exenatide (Byetta)	SC	Twice a day	5 mcg 10 mcg	-Reduced HbA1c level (0.5-1.0%) -Weight reduction -Reduced blood pressure -Low risk of hypoglycemia	FDA
Exenatide long acting release (Byduron)	SC	Once a week	2 mg	-Reduced HbA1c level (1.5-1.9%) -Weight reduction -Low risk of hypoglycemia	FDA
Liraglutide (Victoza)	SC	Once a day	0.6 mg 1.2 mg 1.8 mg	-Reduced HbA1c level (2.5%) -Weight reduction -Low risk of hypoglycemia	FDA
Lixisenatide (Adylxin, Lyxumia)	SC	Once a day	10 mcg 20 mcg	-Reduced HbA1c level (0.5-1.0%)	TGA (Lyxumia) FDA (Adylxin)
Semaglutide	SC	Once a week	0.5 mg 1 mg	Clinical studies are in progress	Not approved
Dulaglutide (Trulicity)	SC	Once a week	1.5 mg	-Reduced HbA1c level (0.78%) -Weight reduction -Low risk of hypoglycemia	FDA
Albiglutide (Tanzeum, Eperzan)	SC	Once a week	30 mg 50 mg	-Reduced HbA1c level (0.78%) -Weight reduction -Low risk of hypoglycemia	FDA

FDA: Food and Drug Administration
TGA: Therapeutic Goods Administration

GLP-1 receptor agonists increase the risk of acute pancreatitis because they stimulate proliferation of pancreatic ductal cells. Therefore they are contraindicated in patients with pancreatic adenocarcinoma. Besides, presence of GLP-1 receptors have been shown on C cell lines in thyroid glands of rodents and humans. Therefore, they are also contraindicated in C cell thyroid cancer. The risk of hypoglycemia is quite low, as they do not directly stimulate insulin secretion; however, hypoglycemia risk is increased when used in combination with other oral insulin secretagogues.

Apart from the pancreas, GLP-1 receptors are also present on cardiac and vascular tissues (Saraiva & Sposito, 2014). Unlike the classical pancreatic type GLP-1 receptors, these receptors mediate various mechanisms other than its effects on insulin and glucagon (Ban et al., 2010; Ban et al., 2008).

Cardiovascular effects of GLP-1 receptor agonists in diabetes

Endothelial and Vascular Effects

Vascular dysfunction occurring at later stages of diabetes may lead to early atherosclerosis. In vascular system, GLP-1 receptors are localized to endothelial cells and smooth muscle cells (Goud et al., 2016). Clinical and experimental studies have shown that GLP-1 and GLP-1 receptors on endothelial cells show direct cardioprotective effect and improve endothelial functions in T2DM and T1DM (Cantini, Mannucci, & Luconi, 2016). In their study with T2DM patients, *Motta et al.* found that exenatide use directly improved GLP-1-mediated endothelial function; indirectly prevented dysfunction due to its blood glucose and lipid lowering effect; and increased endothelial nitric oxide synthase (e-NOS)-mediated nitric oxide (NO) production due to its stimulatory effect on endothelial AMPK pathway activation (Motta, Koska, Reaven, & Migrino, 2012).

Endothelial dysfunction is characterized by increased adhesion molecules and chemokines. Increased number of monocytes and inflammatory cells at the vascular wall causes adhesion (Simo & Hernandez, 2016) and disturbs NO mechanism, resulting in endothelial dysfunction (Goud et al., 2016). GLP-1 improves cardiac functions and cardiac glucose metabolism, and protects cardiac microvessels against oxidative stress, apoptosis, and microvascular barrier dysfunction of diabetes (Saraiva & Sposito, 2014). The protective mechanism of GLP-1 depends on downstream inhibition of rho through cAMP-PKA-

mediated pathway (Saraiva & Sposito, 2014). Animal studies with liraglutide in hyperglycemic environment have shown that it increased NO production via increasing eNOS phosphorylation, inhibited NF-kappaB activation, and augmented anti-inflammatory response in vascular endothelial cells through partial activation of AMPK pathway (Y. Hattori et al., 2010); inhibited upregulation of stress markers that are secreted from the endoplasmic reticulum upon hyperglycemia, and prevented apoptosis through inhibition of mitochondrial degradation (Schisano et al., 2012). *Salheen et al.* found that superoxide production was decreased when rat mesenteric arteries exposed to high dose glucose in in vitro environment were acutely treated with the GLP-1 receptor agonist exendin-4; and NO- and EDHF-mediated endothelial origin relaxation response was improved (Salheen, Panchapakesan, Pollock, & Woodman, 2015). *Wang et al.* found that oxidative stress that is induced by glucose and NADPH oxidase-ROS pathway mediation was suppressed by GLP-1 receptor agonists (R. Wang et al., 2015).

Effects on Blood Pressure

GLP-1 receptor agonists cause a dose-dependent and reversible relaxation in systemic and pulmonary arteries, and regulate blood pressure (Katout et al., 2014). In their clinical study with T2DM patients, *Chaudhuri et al.* observed that exenatide treatment caused profound increase in plasma ANP, cGMP and cAMP levels, and significant reduction in angiotensinogen concentration (Chaudhuri et al., 2016). These findings suggest that exenatide lowers blood pressure by increasing the levels of a group of vasodilator susbtances and suppressing the renin-angiotensin system.

Robinson et al. reported that exenatide caused 2.4 mmHg reduction in systolic blood pressure compared to placebo or insulin (Robinson, Holt, Rees, Randeva, & O'Hare, 2013). *Drucker et al.* studied long-acting and short-acting exenatide treatment in T2DM patients, and they found that long-acting exenatide caused greater reduction in systolic blood pressure (4.7 mmHg) compared to short-acting exenatide (3.4 mmHg), but they did not find any difference between the two forms regarding the reduction in diastolic blood pressure (1.7 mmHg) (Drucker et al., 2008). *Fonseca et al.* administered liraglutide to T2DM patients with two different doses, and they found liraglutide dose-dependent reduction in systolic blood pressure (1.2 mg (2.7 mmHg); 1.8 mg (2.9 mmHg)) (Fonseca et al., 2014). *Marso et al.* administered liraglutide treatment to T2DM patients with HbA1c levels >7% and they reported 1.2 mmHg reduction in systolic blood pressure and 0.6 mmHg reduction in diastolic blood pressure (Marso et al., 2016).

All these data suggest that GLP-1 receptor agonists exert their effect on blood pressure via several mechanisms including inhibition of sodium absorption at the proximal tubule, central effects of GLP-1 receptor agonists (Scheen, 2015), atrial natriuretic peptide (ANP) secretion and vasodilatation through atrial GLP-1 receptor mediated cAMP-EPAC-ANP pathways (M. Kim et al., 2013), smooth muscle relaxation and NO-independent cGMP (cyclic guanosine monophosphate) mediated vasodilatation (Jensen et al., 2015), NO-mediated relaxation, and weight reduction due to delayed gastric emptying and suppressed appetite (Chaudhuri et al., 2016).

Cardio-Renal Effects

GLP-1 receptors are thought to have antihypertensive effect through kidney-heart-renal system. However, this mechanism has not been clearly understood yet. *Kim et al.* reported that Rap guanine nucleotide exchange factor translocation to the membrane is triggered by activation of GLP-1 receptors in the atrium of the heart, and that this mechanism caused increased ANP release, resulting in natriuresis, cGMP-mediated smooth muscle relaxation and reduced blood pressure (M. Kim et al., 2013). However, one year later, in their study including healthy male individuals, *Skov et al.* reported that although 2 hours of GLP infusion significantly increased urinary sodium excretion, it did not result in a significant change in proANP concentrations, and when the GLP-1 infusion was stopped, sodium excretion was decreased but proANP concentration was unaltered. Based on these findings, they proposed that natriuretic effect of GLP-1 is not related to ANP (Skov, Holst, Gotze, Frokiaer, & Christiansen, 2014).

Cardiac Effects

The most important cardiovascular adverse effect observed at later stages of diabetes is diabetic cardiomyopathy that is manifested by ischemic cardiomyopathy (Gelzinis, 2014). GLP-1 receptor agonists are thought to influence cardiovascular system through several distinct mechanisms.

GLP-1 agonists, which show inotropic effect on the heart, are believed to ameliorate left ventricular structure and functions that are disturbed because of diabetes. Clinical studies on patients with heart failure have shown that exenatide increases left ventricular ejection fraction and heart rate (Mikhail, 2014), increased number of GLP-1 receptors on the heart and increased plasma GLP-1 concentration improve

left ventricular function and preserve left ventricular fraction (A. Hattori et al., 2013). In a meta-analysis including 32 studies on T2DM patients, *Robinson et al.* reported that GLP-1 analogs accelerated the heart rate by 1.86 bpm, and that this effect was more pronounced with liraglutide and long-acting exenatide compared to short-acting exenatide. Similarly, in another meta-analysis by *Katout et al.* which included 33 studies, GLP-1 agonists (liraglutide and exenatide) were reported to have chronotropic effect, increasing the heart rate by 1.30 bpm (Katout et al., 2014; Robinson et al., 2013). *Lorenz et al*. found that long-acting exenatide, liraglutide, albuglutide and dulaglutide caused more pronounced and sustained increase in the heart rate compared to short-acting exenatide and lixinatide; and they stated that this increase might be related to GLP-1's direct effect on myocytes found in the sinoatrial node of the heart, or to sympathetic nervous system activation (Lorenz et al., 2017). *Marso et al*. reported that liraglutide treatment in T2DM patients with HbA1c levels >7% resulted in significant reduction in cardiovascular mortality and incidence of microvascular adverse events such as nephropathy and retinopathy (Marso et al., 2016).

The protective effect of GLP-1 receptors that are found in the atrium of the heart and sinoatrial node on left ventricular structure and function can be explained via several mechanisms. First, GLP-1 receptor agonists reduce multiple prosurvival kinases that cause cardiac damage following activation after ischemia. Two different studies on this topic have shown that activation of GLP-1 reduced the levels of kinases including cAMP inhibitor, PI3 kinase inhibitor, p42/44 mitogen activated protein kinase inhibitor and N-Ac-GLP-1(7-34)amide in the rat heart (Bose, Mocanu, Carr, Brand, & Yellon, 2005; Salling, Dohler, Engstrom, & Treiman, 2012).

Second, GLP-1 receptor agonists can increase energy production. In their study including obese and T2DM patients, *Moberly et al.* found that the GLP-1 increased myocardial glucose uptake (Moberly et al., 2013).

As the third mechanism, GLP-1 receptors found on the heart activate adenylate cyclase-mediated cAMP. *Kuna et al.* observed GLP-1 receptor/ligand complex and adenylate cyclase in endosome (Kuna et al., 2013). *Vila Petroff et al.* studied murine cardiomyocytes with no contractility induction and no increase in intracellular Ca^{+2}, and they showed that GLP-1 treatment resulted in increased cAMP levels (Vila Petroff, Egan, Wang, & Sollott, 2001). Later studies confirmed that GLP-1 mediated activation of cAMP/PKA pathway reduced cardiac myocyte apoptosis and stimulated generation of cardiomyocytes, and provided regulation of glucose metabolism through eNOS activation (Aravindhan et al., 2015; Esposito et al., 2016; D. Wang et al., 2013; Xiao, Nikolskaya, Jaye, & Sigg, 2011) .

As another important mechanism, GLP-1 receptor activation suppresses the activity of NADPH oxidase which is an effective reactive oxygen molecule, and increases energy use by preventing glucotoxicity (Poudyal, 2016). *Inou et al.* found that liraglutide treatment in streptozotocin-induced diabetic rats showed ameliorating effect on oxidative stress caused by cardiac steatosis and DAG-PKC-NAD(P)H pathway/AMPK-Sirt1 pathways activation, independent of its glucose-lowering effect (Inoue et al., 2015). It was found that in adult heart incubated in a high-glucose environment; GLP-1 induces α2-AMPK activation; prevents p47phox translocation which plays role in NADPH oxidase activity and is increased in high glucose; and inhibits activation of NOX2 activation which is transmembrane oxidase component of NADPH; and therefore, suppresses production of reactive oxygen species (Balteau et al., 2014; El-Benna, Dang, Gougerot-Pocidalo, Marie, & Braut-Boucher, 2009). In summary, GLP-1 and GLP-1 receptor agonists have been associated with cardioprotective effects, reduced damage after ischemia, increased efficiency of energy substrate utilization, and reduced oxidative stress/damage.

DPP-4 inhibitors

In T2DM patients, impaired incretin response, DPP-4 enzyme activity and glucose homeostasis are related with each other. Increased HbA1c level plays role in diminished incretin response by increasing DPP-4 activity (Mannucci et al., 2005).

DPP-4 inhibitors cause 80% inhibition of the dipeptidyl peptidase-4 (DPP-4), an enzyme whose expression is increased in T2DM, and they prolong the time during which endogenous GLP-1 and GIP are maintained at active concentrations in the circulation by 2-3 folds (Cuny, Guerci, & Cariou, 2012). As its duration of action is prolonged, endogenous GLP-1 increases insulin release from the pancreas and suppresses glucagon release. While they increase the number of pancreatic β-cells, they also protect them against apoptosis. They reduce postprandial glucose levels and prevent sudden fluctuations in glucose levels. Clinical studies have shown that they reduce HbA1c levels by 0.5%-1% (Jayawardene et al., 2014; Jose & Inzucchi, 2012).

Because they cannot increase blood GLP-1 level as GLP-1 receptor agonists (Waldrop et al., 2016), they cannot slow down gastric emptying time, and

their effect on body weight is neutral. They are not as effective as GLP-1 receptor agonists on insulin secretion; however, their gastrointestinal side effects are milder. Furthermore, patient compliance is higher compared to GLP-1 receptor agonists because they are taken via oral route. Additionally, the risk of hypoglycemia is lower.

This group of drugs are also contraindicated in pancreatitis and pancreas cancer. Additionally, it has been reported that headache, nasopharyngitis, upper respiratory tract infection, urinary system infection could develop due to suppression of the enzyme DPP-4, however, these side effects were not frequently encountered in the clinical practice (Jayawardene et al., 2014; Willemen et al., 2011).

Table 2 shows existing DPP-4 inhibitors that are designed for clinical use. The first approved drug in this group was sitagliptin. Drugs that were introduced to the market afterwards were vildagliptin, saxagliptin, alogliptin and linagliptin, in order of introduction (Scheen, 2012). Except vildagliptin, all DPP-4 inhibitors are used once a day. Due to their advantages such as no weight gain, good tolerability, and low hypoglycemia risk, they are preferred in treatment of T2DM patients who are elderly, or have renal failure or cardiovascular complication, in combination with an antihyperglycemic agent from a different group. (Hinnen, 2015; Scott, 2017).

Table 2: DPP-4 inhibitors

	Administration route	Dose interval	Dose amount	Advantages	**Approval**
Sitagliptin (Januvia, Galactiv, Tesavel, Xelevia)	Oral	Once a day	100 mg, 50 mg, 25 mg	-Reduced HbA1c (1.1%) -Low hypoglycemia risk -No gain weight	FDA
Vildagliptin (Galvus)	Oral	Twice a day	50 mg	-Reduced HbA1c (1.1%) -Low hypoglycemia risk	FDA
Saxagliptin (Onglyza)	Oral	Once a day	5 mg	-Reduced HbA1c (0.8%) -Low hypoglycemia risk	FDA
Linagliptin (Trajenta)	Oral	Once a day	5 mg	-Reduced HbA1c (0.64%)	FDA
Alogliptin (Nesina)	Oral	Once a week	6,25 mg, 12,5 mg, 25 mg	-Reduced HbA1c (0.8%)	Not approved

FDA: Food and Drug Administration

As with GLP-1 receptor agonists, increased GLP-1 through DPP-4 inhibitors is thought to show direct or indirect protective action in cardiovascular system of diabetic patients by stimulating GLP-1 receptors on heart and vascular tissue.

Endothelial and Vascular Effects

DPP-4 enzyme is predominantly localized to endothelial cells of the microvascular circulation (Fadini, Albiero, & Avogaro, 2015). DPP-4 inhibitors display protective effect on the vascular by either directly through inhibition of these enzymes or by GLP-1 mediated mechanisms. Moreover, this effect is also observed in patients without cardiovascular complications. *Widlansky et al.* reported that DPP-4 inhibitors reduced vascular adhesion, improved endothelial functions and reduced cardiovascular disease in T2DM patients who did not develop heart failure or renal failure (Widlansky et al., 2017).

Inhibition of the DPP-4 enzyme in endothelial cells are believed to have direct contribution in the vasodilation (Scheen, 2013; Widlansky et al., 2017). Studies on saxagliptin (Mason et al., 2012) and sitagliptin (Liu et al., 2012) have shown that increased GLP-1 and GLP-1 receptor expressions cause endothelial NO consumption as a result of cAMP mediated protein kinase A activation. In their in vitro study with aorta incubated with vasoconstrictor substances, *Shah et al.* found that inhibition of endothelial DPP-4 with alogliptin resulted in activation of Src-Akt-eNOS mediated NO release and vascular potassium channels, leading to rapid vascular relaxation response (Shah et al., 2011).

However, the degradation product of GLP-1 by DPP-4, GLP-1 (9-36) has been shown to contribute in the vasodilatation response (Basu et al., 2007). In that case, it should be kept in mind that inhibition of DPP-4 enzyme may also cause reduction of vasodilatation response.

Studies examining the effects of sitagliptin on endothelial functions have contradicting

results. *Matsubara et al.* found that apart from its hypoglycemic effect, sitagliptin treatment in diabetic patients with cardiac artery impairment improved endothelial function and reduced inflammation (Matsubara et al., 2013), whereas *Hage et al.* reported no significant improvement in the complications with sitagliptin treatment in diabetic patients with acute coronary syndrome (Hage et al., 2014).

Studies indicate treatment with DPP-4 inhibitors cause reduction of biomarkers responsible of inflammation, and oxidative stress. *Ta et al.* showed that in apolipoprotein E (apoE)-deficient mice, aligliptine treatment inhibited IL-6 and IL-1β upregulation and prevented atherosclerotic plaque formation (Ta, Schuyler, Li, Lopes-Virella, & Huang, 2011). *Terasaki et al.* found that the antiatherosclerotic effect of vildagliptin in apoE-deficient mice was due to inhibition of macrophage proliferation and that this effect was independent from GLP-1 action (Terasaki et al., 2013). *Akita et al.* reported that alogliptin reduced arterial inflammation and intimal thickening (Akita, Isoda, Shimada, & Daida, 2015). *Takai et al.* stated that sitagliptin and linagliptin treatment in diabetic rats inhibited Rac activation, suppressed oxidative stress, chemokine production and monocyte clustering, and augmented vasodilatation responses. Moreover, linagliptin was found to be more effective regarding vascular protection mechanism (Takai, Sakonjo, & Jin, 2014). *Hu et al.* found that sitagliptin acted by inhibiting induction of plasminogen activator inhibitor type 1 (PAI-1), intracellular adhesion molecule-1 (ICAM-1) and tumor necrosis factor alpha (TNFa) (Hu, Liu, Simpson, & Dear, 2013); *Khan et al.* found that vildagliptin improved fibrinolysis and coagulation cascade (Khan, Khan, Panda, Akhtar, & Najmi, 2015).

Effects on Blood Pressure

DPP-4 inhibitors cause small but significant reduction in the blood pressure. *Yamamoto et al* reported that GLP-1 receptor stimulation augmented by inhibition of DPP-4 due to activation of autonomic regulatory neurons increased blood pressure and heart rate (Yamamoto et al., 2002). *Shah et al.* showed that DPP-4 inhibitors caused relaxed vascular tonus as a result of NO-mediated peripheral vasodilatation and reduced peripheral vascular resistance, and that this response contributed in controlling the blood pressure (Shah et al., 2011).

In fact, the effect of DPP-4 inhibitors on blood pressure has not been truly confirmed clinically. Clinical studies on T2DM patients have shown that addition of saxagliptin to metformin or gliburide resulted in a reduction in systolic and diastolic blood pressure that was not statistically significant (Chacra et al., 2009; Goke et al., 2010). Clinical studies on T2DM patients have shown that sitagliptin caused a small decrease in the systolic blood pressure (Koren et al., 2012; Ogawa et al., 2011). Although *Bosi et al.* found that 24-week 100 mg/day vildagliptin treatment in T2DM patients caused significant reduction in diastolic blood pressure (0.3 mmHg); in another study, *Dejager et al.* did not find significant difference in systolic blood pressure when 24-week vildagliptin treatment at different doses (50 mg once daily, 50 mg BID, 100 mg once daily) was compared to placebo in T2DM patients (Bosi, Camisasca, Collober, Rochotte, & Garber, 2007; Dejager, Razac, Foley, & Schweizer, 2007).

In general, DPP-4 inhibitors contribute in blood pressure control in T2DM patients by lowering systolic blood pressure by 0-5 mmHg and diastolic blood pressure by 0-3 mmHg.

Cardio-Renal Effects

DPP-4 enzyme is present on the capillary endothelial cells of the renal system, and on brush border membrane of proximal tubule cells, along with Na+/H+ exchanger-3. DPP-4 is increased in obesity/overfeeding and inflammatory states (Aroor, Sowers, Jia, & DeMarco, 2014). DPP-4 inhibitors attenuate renal damage, and indirectly improve cardiovascular complications. *Chavkovska et al.* showed that DPP-4 inhibitors improved cardiac fibrosis by reducing cardiac matrix proteins and B-type natriuretic peptide (BNP) mRNA levels in uremic cardiomyopathy model (Chaykovska et al., 2011).

Cardiac Effects

Three large clinical trials have been conducted in order to investigate cardiovascular safety of DPP-4 inhibitors in T2DM patients: (SAVOR-TIMI 53 (Saxagliptin and Cardiovascular Outcomes in Patients with Type 2 Diabetes Mellitus) (Scirica et al., 2013), EXAMINE (Examination of Cardiovascular Outcomes with Alogliptin versus Standart of Care) (White et al., 2013), and TECOS (Trial Evaluating Cardiovascular Outcomes with Sitagliptin)(Green et al., 2015)). TECOS trial did not find any significant increase or decrease in cardiovascular complications (cardivascular mortality, myocardial infarction, ischemic stroke, heart failure). Unexpectedly, SAVOR-TIMI53 trial found increase risk of hospitalization rate for heart failure with saxagliptin (3,5% for saxagliptin vs 2,8% for placebo) (Scirica et al., 2013). Additional studies have concluded that the possible association between saxagliptin use and heart failure was more

pronounced among patients with and without history of chronic renal failure and heart failure, and increased levels of natriuretic peptide (Scirica et al., 2015). This effect was not observed in EXAMINE that was conducted with alogliptin. But in a post subgroup analysis motivated partially by the results of the SAVOR, alogliptin use was associated with an increased risk of hospitalization for heart failure among patients without history of hearth failure (2,2% for alogliptin vs 1,3 for placebo). Interestingly this risk was not present among patients with prior hearth failure (White et al., 2013).

Similar to these clinical trials, different meta-analyses involving T2DM patients did not find significant difference between DPP-4 inhibitor and placebo groups regarding mortality rate and incidence of acute coronary syndrome or stroke (J. Y. Kim, Yang, Lee, & Chang, 2016; Wu, Hopper, Skiba, & Krum, 2014). Unlike other studies, *Savarese et al.* reported that DPP-4 inhibitors did not have any effect on cardiovascular mortality in diabetic patients; and although they reduced the risk of myocardial infarction in the short-term, this effect could not be observed in the long term, and long-term DPP-4 inhibitors use was associated with the risk of heart failure (Savarese et al., 2015). Meta-analyses that investigated DPP-4 inhibitors on their own have shown that vildagliptin (McInnes et al., 2015) and linagliptin (Rosenstock et al., 2015) did not increase the risk for major acute cardiovascular side effects and heart failure. In conclusion, DPP-4 inhibitors except alogliptin and saxagliptin do not increase the risk of heart failure or incidence of major adverse cardiovascular events.

Existing evidence support anti-atherosclerotic effect of DPP-4 inhibitors. In two studies on T2DM patients without history of cardiovascular disease, *Mita et al.* showed that sitagliptin (Mita, Katakami, Shiraiwa, et al., 2016) and alogliptin (Mita, Katakami, Yoshii, et al., 2016) treatment were more effective in prevention from carotid atherosclerosis compared to the conventional treatments.

Finally, animal studies using DPP-4 inhibitors in non-diabetic animals have shown that DPP-4 inhibitors displayed a pleotropic effect and contributed in improvement of left ventricular functions that were disturbed because of ischemia/reperfusion injury, and reduced infarction size (Huisamen, Genis, Marais, & Lochner, 2011; Sauve et al., 2010).

CONCLUSION

The main purpose of the life-long treatment in diabetic patients is to achieve a good glycemic control and cardiovascular protection without triggering hypoglycemia or cardiovascular complications. However, achieving blood glucose homeostasis without inducing cardiovascular adverse effects is not always possible with the conventional antihyperglycemic agents. In addition their blood glucose lowering effect, incretin mimetic drugs reduce cardiovascular complications and therefore have significance as new generation antihyperglycemic agents. They act either directly or indirectly on vascular endothelium, atherosclerosis, heart failure, blood pressure, lipid metabolism and body weight. Although they are not as effective as classical antidiabetic agents regarding their blood glucose lowering effect, when used in combination, they may reduce cardiovascular complications that are caused by the other agents. However, although these effects have been demonstrated in preclinical studies, more and broad pre-clinical and clinical studies are necessary for fully understanding the interaction of GLP-1 receptor agonists and DPP-4 inhibitors with the cardiovascular system.

REFERENCES

Akita, K., Isoda, K., Shimada, K., & Daida, H. (2015). 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, 4*(3), e001469. doi:10.1161/JAHA.114.001469

Aravindhan, K., Bao, W., Harpel, M. R., Willette, R. N., Lepore, J. J., & Jucker, B. M. (2015). Cardioprotection Resulting from Glucagon-Like Peptide-1 Administration Involves Shifting Metabolic Substrate Utilization to Increase Energy Efficiency in the Rat Heart. *PLoS One, 10*(6), e0130894. doi:10.1371/journal.pone.0130894

Aroor, A. R., Sowers, J. R., Jia, G., & DeMarco, V. G. (2014). Pleiotropic effects of the dipeptidylpeptidase-4 inhibitors on the cardiovascular system. *Am J Physiol Heart Circ Physiol, 307*(4), H477-492. doi:10.1152/ajpheart.00209.2014

Balteau, M., Van Steenbergen, A., Timmermans, A. D., Dessy, C., Behets-Wydemans, G., Tajeddine, N., . . . Beauloye, C. (2014). AMPK activation by glucagon-like peptide-1 prevents NADPH oxidase activation induced by hyperglycemia in adult cardiomyocytes. *Am J Physiol Heart Circ Physiol, 307*(8), H1120-1133. doi:10.1152/ajpheart.00210.2014

Ban, K., Kim, K. H., Cho, C. K., Sauve, M., Diamandis, E. P., Backx, P. H., . . . Husain, M. (2010). Glucagon-like peptide (GLP)-1(9-36)amide-mediated cytoprotection is blocked by exendin(9-39) yet does not require the known GLP-1 receptor. *Endocrinology, 151*(4), 1520-1531. doi:10.1210/en.2009-1197

Ban, K., Noyan-Ashraf, M. H., Hoefer, J., Bolz, S. S., Drucker, D. J., & Husain, M. (2008). Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. *Circulation, 117*(18), 2340-2350. doi:10.1161/CIRCULATIONAHA.107.739938

Basu, A., Charkoudian, N., Schrage, W., Rizza, R. A., Basu, R., & Joyner, M. J. (2007). Beneficial effects of GLP-1 on endothelial function in humans: dampening by glyburide but not by glimepiride. *Am J Physiol Endocrinol Metab, 293*(5), E1289-1295. doi:10.1152/ajpendo.00373.2007

Bose, A. K., Mocanu, M. M., Carr, R. D., Brand, C. L., & Yellon, D. M. (2005). Glucagon-like peptide 1 can directly protect the heart against ischemia/reperfusion injury. *Diabetes, 54*(1), 146-151.

Bosi, E., Camisasca, R. P., Collober, C., Rochotte, E., & Garber, A. J. (2007). Effects of vildagliptin on glucose control over 24 weeks in patients with type 2 diabetes inadequately controlled with metformin. *Diabetes Care, 30*(4), 890-895. doi:10.2337/dc06-1732

Burgmaier, M., Heinrich, C., & Marx, N. (2013). Cardiovascular effects of GLP-1 and GLP-1-based therapies: implications for the cardiovascular continuum in diabetes? *Diabet Med, 30*(3), 289-299. doi:10.1111/j.1464-5491.2012.03746.x

Cantini, G., Mannucci, E., & Luconi, M. (2016). Perspectives in GLP-1 Research: New Targets, New Receptors. *Trends Endocrinol Metab, 27*(6), 427-438. doi:10.1016/j.tem.2016.03.017

Chacra, A. R., Tan, G. H., Apanovitch, A., Ravichandran, S., List, J., Chen, R., & Investigators, C. V. (2009). Saxagliptin added to a submaximal dose of sulphonylurea improves glycaemic control compared with uptitration of sulphonylurea in patients with type 2 diabetes: a randomised controlled trial. *Int J Clin Pract, 63*(9), 1395-1406. doi:10.1111/j.1742-1241.2009.02143.x

Chaudhuri, A., Ghanim, H., Makdissi, A., Green, K., Abuaysheh, S., Batra, M., . . . Dandona, P. (2016). Exenatide Induces an Increase in Vasodilatory and a Decrease in Vasoconstrictive Mediators. *Diabetes Obes Metab*. doi:10.1111/dom.12835

Chaykovska, L., von Websky, K., Rahnenfuhrer, J., Alter, M., Heiden, S., Fuchs, H., . . . Hocher, B. (2011). Effects of DPP-4 inhibitors on the heart in a rat model of uremic cardiomyopathy. *PLoS One, 6*(11), e27861. doi:10.1371/journal.pone.0027861

Chong, C. R., Clarke, K., & Levelt, E. (2017). Metabolic Remodeling in Diabetic Cardiomyopathy. *Cardiovasc Res*. doi:10.1093/cvr/cvx018

Cuny, T., Guerci, B., & Cariou, B. (2012). New avenues for the pharmacological management of type 2 diabetes: an update. *Ann Endocrinol (Paris), 73*(5), 459-468. doi:10.1016/j.ando.2012.09.002

Dailey, M. J., & Moran, T. H. (2013). Glucagon-like peptide 1 and appetite. *Trends Endocrinol Metab, 24*(2), 85-91. doi:10.1016/j.tem.2012.11.008

Dejager, S., Razac, S., Foley, J. E., & Schweizer, A. (2007). Vildagliptin in drug-naive patients with type 2 diabetes: a 24-week, double-blind, randomized, placebo-controlled, multiple-dose study. *Horm Metab Res, 39*(3), 218-223. doi:10.1055/s-2007-970422

Drucker, D. J., Buse, J. B., Taylor, K., Kendall, D. M., Trautmann, M., Zhuang, D., . . . Group, D.-S. (2008). Exenatide once weekly versus twice daily for the treatment of type 2 diabetes: a randomised, open-label, non-inferiority study. *Lancet, 372*(9645), 1240-1250. doi:10.1016/S0140-6736(08)61206-4

Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. (1998). *Lancet, 352*(9131), 854-865.

El-Benna, J., Dang, P. M., Gougerot-Pocidalo, M. A., Marie, J. C., & Braut-Boucher, F. (2009). p47phox, the phagocyte NADPH oxidase/NOX2 organizer: structure, phosphorylation and implication in diseases. *Exp Mol Med, 41*(4), 217-225. doi:10.3858/emm.2009.41.4.058

Esposito, G., Cappetta, D., Russo, R., Rivellino, A., Ciuffreda, L. P., Roviezzo, F., . . . Urbanek, K. (2016). Dipeptidyl peptidase 4 inhibitor sitagliptin reduces inflammation, fibrosis and preserves diastolic function in a rat model of heart failure with preserved ejection fraction. *Br J Pharmacol*. doi:10.1111/bph.13686

Fadini, G. P., Albiero, M., & Avogaro, A. (2015). Direct effects of DPP-4 inhibition on the vasculature. Reconciling basic evidence with lack of clinical evidence. *Vascul Pharmacol, 73*, 1-3. doi:10.1016/j.vph.2015.08.004

Fisman, E. Z., & Tenenbaum, A. (2015). Antidiabetic treatment with gliptins: focus on cardiovascular effects and outcomes. *Cardiovasc Diabetol, 14*, 129. doi:10.1186/s12933-015-0294-0

Fonseca, V. A., Devries, J. H., Henry, R. R., Donsmark, M., Thomsen, H. F., & Plutzky, J. (2014). Reductions in systolic blood pressure with liraglutide in patients with type 2 diabetes: insights from a patient-level pooled analysis of six randomized clinical trials. *J Diabetes Complications, 28*(3), 399-405. doi:10.1016/j.jdiacomp.2014.01.009

Gelzinis, T. A. (2014). New Insights Into Diastolic Dysfunction and Heart Failure With Preserved Ejection Fraction. *Semin Cardiothorac Vasc Anesth, 18*(2), 208-217. doi:10.1177/1089253213510748

Goke, B., Gallwitz, B., Eriksson, J., Hellqvist, A., Gause-Nilsson, I., & Investigators, D. C. (2010). Saxagliptin is non-inferior to glipizide in patients with type 2 diabetes mellitus inadequately controlled on metformin alone: a 52-week randomised controlled trial. *Int J Clin Pract, 64*(12), 1619-1631. doi:10.1111/j.1742-1241.2010.02510.x

Goud, A., Zhong, J., Peters, M., Brook, R. D., & Rajagopalan, S. (2016). GLP-1 Agonists and Blood Pressure: A Review of the Evidence. *Curr Hypertens Rep, 18*(2), 16. doi:10.1007/s11906-015-0621-6

Graham, D. J., Ouellet-Hellstrom, R., MaCurdy, T. E., Ali, F., Sholley, C., Worrall, C., & Kelman, J. A. (2010). Risk of acute myocardial infarction, stroke, heart failure, and death in elderly Medicare patients treated with rosiglitazone or pioglitazone. *JAMA, 304*(4), 411-418. doi:10.1001/jama.2010.920

Green, J. B., Bethel, M. A., Armstrong, P. W., Buse, J. B., Engel, S. S., Garg, J., . . . Group, T. S. (2015). Effect of Sitagliptin on Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med, 373*(3), 232-242. doi:10.1056/NEJMoa1501352

Hage, C., Brismar, K., Lundman, P., Norhammar, A., Ryden, L., & Mellbin, L. (2014). 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, 11*(4), 290-293. doi:10.1177/1479164114533355

Hattori, A., Kawamura, I., Yamada, Y., Kanamori, H., Aoyama, T., Ushikoshi, H., . . . Minatoguchi, S. (2013). Elevated plasma GLP-1 levels and enhanced expression of cardiac GLP-1 receptors as markers of left ventricular systolic dysfunction: a cross-sectional study. *BMJ Open, 3*(9), e003201. doi:10.1136/bmjopen-2013-003201

Hattori, Y., Jojima, T., Tomizawa, A., Satoh, H., Hattori, S., Kasai, K., & Hayashi, T. (2010). A glucagon-like peptide-1 (GLP-1) analogue, liraglutide, upregulates nitric oxide production and exerts anti-inflammatory action in endothelial cells. *Diabetologia, 53*(10), 2256-2263. doi:10.1007/s00125-010-1831-8

Hinnen, D. (2015). Dipeptidyl Peptidase-4 Inhibitors in Diverse Patient Populations With Type 2 Diabetes. *Diabetes Educ, 41*(1 Suppl), 19S-31S. doi:10.1177/0145721715609420

Holman, R. R., Paul, S. K., Bethel, M. A., Matthews, D. R., & Neil, H. A. (2008). 10-year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med, 359*(15), 1577-1589. doi:10.1056/NEJMoa0806470

Hu, Y., Liu, H., Simpson, R. W., & Dear, A. E. (2013). GLP-1-dependent and independent effects and molecular mechanisms of a dipeptidyl peptidase 4 inhibitor in vascular endothelial cells. *Mol Biol Rep, 40*(3), 2273-2279. doi:10.1007/s11033-012-2290-8

Huisamen, B., Genis, A., Marais, E., & Lochner, A. (2011). Pre-treatment with a DPP-4 inhibitor is infarct sparing in hearts from obese, pre-diabetic rats. *Cardiovasc Drugs Ther, 25*(1), 13-20. doi:10.1007/s10557-010-6271-7

Inoue, T., Inoguchi, T., Sonoda, N., Hendarto, H., Makimura, H., Sasaki, S., . . . Takayanagi, R. (2015). GLP-1 analog liraglutide protects against cardiac steatosis, oxidative stress and apoptosis in streptozotocin-induced diabetic rats. *Atherosclerosis, 240*(1), 250-259. doi:10.1016/j.atherosclerosis.2015.03.026

Jayawardene, D., Ward, G. M., O'Neal, D. N., Theverkalam, G., MacIsaac, A. I., & MacIsaac, R. J. (2014). New treatments for type 2 diabetes: cardiovascular protection beyond glucose lowering? *Heart Lung Circ, 23*(11), 997-1008. doi:10.1016/j.hlc.2014.05.007

Jensen, E. P., Poulsen, S. S., Kissow, H., Holstein-Rathlou, N. H., Deacon, C. F., Jensen, B. L., . . . Sorensen, C. M. (2015). Activation of GLP-1

receptors on vascular smooth muscle cells reduces the autoregulatory response in afferent arterioles and increases renal blood flow. *Am J Physiol Renal Physiol, 308*(8), F867-877. doi:10.1152/ajprenal.00527.2014

Jose, T., & Inzucchi, S. E. (2012). Cardiovascular effects of the DPP-4 inhibitors. *Diab Vasc Dis Res, 9*(2), 109-116. doi:10.1177/1479164111436236

Katout, M., Zhu, H., Rutsky, J., Shah, P., Brook, R. D., Zhong, J., & Rajagopalan, S. (2014). Effect of GLP-1 mimetics on blood pressure and relationship to weight loss and glycemia lowering: results of a systematic meta-analysis and meta-regression. *Am J Hypertens, 27*(1), 130-139. doi:10.1093/ajh/hpt196

Khan, S., Khan, S., Panda, B. P., Akhtar, M., & Najmi, A. K. (2015). Potential effects of vildagliptin on biomarkers associated with prothrombosis in diabetes mellitus. *Expert Opin Ther Targets, 19*(12), 1607-1616. doi:10.1517/14728222.2016.1086338

Kim, J. Y., Yang, S., Lee, J. I., & Chang, M. J. (2016). Cardiovascular Effect of Incretin-Based Therapy in Patients with Type 2 Diabetes Mellitus: Systematic Review and Meta-Analysis. *PLoS One, 11*(4), e0153502. doi:10.1371/journal.pone.0153502

Kim, M., Platt, M. J., Shibasaki, T., Quaggin, S. E., Backx, P. H., Seino, S., . . . Drucker, D. J. (2013). GLP-1 receptor activation and Epac2 link atrial natriuretic peptide secretion to control of blood pressure. *Nat Med, 19*(5), 567-575. doi:10.1038/nm.3128

Koren, S., Shemesh-Bar, L., Tirosh, A., Peleg, R. K., Berman, S., Hamad, R. A., . . . Efrati, S. (2012). The effect of sitagliptin versus glibenclamide on arterial stiffness, blood pressure, lipids, and inflammation in type 2 diabetes mellitus patients. *Diabetes Technol Ther, 14*(7), 561-567. doi:10.1089/dia.2011.0296

Kottenberg, E., Thielmann, M., Kleinbongard, P., Frey, U. H., Heine, T., Jakob, H., . . . Peters, J. (2014). Myocardial protection by remote ischaemic pre-conditioning is abolished in sulphonylurea-treated diabetics undergoing coronary revascularisation. *Acta Anaesthesiol Scand, 58*(4), 453-462. doi:10.1111/aas.12278

Kuna, R. S., Girada, S. B., Asalla, S., Vallentyne, J., Maddika, S., Patterson, J. T., . . . Mitra, P. (2013). Glucagon-like peptide-1 receptor-mediated endosomal cAMP generation promotes glucose-stimulated insulin secretion in pancreatic beta-cells. *Am J Physiol Endocrinol Metab, 305*(2), E161-170. doi:10.1152/ajpendo.00551.2012

Little, T. J., Pilichiewicz, A. N., Russo, A., Phillips, L., Jones, K. L., Nauck, M. A., . . . Feinle-Bisset, C. (2006). Effects of intravenous glucagon-like peptide-1 on gastric emptying and intragastric distribution in healthy subjects: relationships with postprandial glycemic and insulinemic responses. *J Clin Endocrinol Metab, 91*(5), 1916-1923. doi:10.1210/jc.2005-2220

Liu, L., Liu, J., Wong, W. T., Tian, X. Y., Lau, C. W., Wang, Y. X., . . . Huang, Y. (2012). Dipeptidyl peptidase 4 inhibitor sitagliptin protects endothelial function in hypertension through a glucagon-like peptide 1-dependent mechanism. *Hypertension, 60*(3), 833-841. doi:10.1161/HYPERTENSIONAHA.112.195115

Lorenz, M., Lawson, F., Owens, D., Raccah, D., Roy-Duval, C., Lehmann, A., . . . Blonde, L. (2017). Differential effects of glucagon-like peptide-1 receptor agonists on heart rate. *Cardiovasc Diabetol, 16*(1), 6. doi:10.1186/s12933-016-0490-6

Lu, C. J., Sun, Y., Muo, C. H., Chen, R. C., Chen, P. C., & Hsu, C. Y. (2013). Risk of stroke with thiazolidinediones: a ten-year nationwide population-based cohort study. *Cerebrovasc Dis, 36*(2), 145-151. doi:10.1159/000353679

Mannucci, E., Pala, L., Ciani, S., Bardini, G., Pezzatini, A., Sposato, I., . . . Rotella, C. M. (2005). Hyperglycaemia increases dipeptidyl peptidase IV activity in diabetes mellitus. *Diabetologia, 48*(6), 1168-1172. doi:10.1007/s00125-005-1749-8

Marso, S. P., Daniels, G. H., Brown-Frandsen, K., Kristensen, P., Mann, J. F., Nauck, M. A., . . . Investigators, L. T. (2016). Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med, 375*(4), 311-322. doi:10.1056/NEJMoa1603827

Mason, R. P., Jacob, R. F., Kubant, R., Ciszewski, A., Corbalan, J. J., & Malinski, T. (2012). Dipeptidyl peptidase-4 inhibition with saxagliptin enhanced nitric oxide release and reduced blood pressure and sICAM-1 levels in hypertensive rats. *J Cardiovasc Pharmacol, 60*(5), 467-473. doi:10.1097/FJC.0b013e31826be204

Matsubara, J., Sugiyama, S., Akiyama, E., Iwashita, S., Kurokawa, H., Ohba, K., . . . Ogawa, H. (2013). 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, 77*(5), 1337-1344.

McInnes, G., Evans, M., Del Prato, S., Stumvoll, M., Schweizer, A., Lukashevich, V., . . . Kothny, W. (2015). Cardiovascular and heart failure safety profile of vildagliptin: a meta-analysis of 17 000 patients. *Diabetes Obes Metab, 17*(11), 1085-1092. doi:10.1111/dom.12548

Mikhail, N. (2014). Effects of incretin-based therapy in patients with heart failure and myocardial infarction. *Endocrine, 47*(1), 21-28. doi:10.1007/s12020-014-0175-4

Mita, T., Katakami, N., Shiraiwa, T., Yoshii, H., Onuma, T., Kuribayashi, N., . . . Collaborators on the Sitagliptin Preventive Study of Intima-Media Thickness Evaluation, T. (2016). Sitagliptin Attenuates the Progression of Carotid Intima-Media Thickening in Insulin-Treated Patients With Type 2 Diabetes: The Sitagliptin Preventive Study of Intima-Media Thickness Evaluation (SPIKE): A Randomized Controlled Trial. *Diabetes Care, 39*(3), 455-464. doi:10.2337/dc15-2145

Mita, T., Katakami, N., Yoshii, H., Onuma, T., Kaneto, H., Osonoi, T., . . . Collaborators on the Study of Preventive Effects of Alogliptin on Diabetic Atherosclerosis, T. (2016). 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, 39*(1), 139-148. doi:10.2337/dc15-0781

Moberly, S. P., Mather, K. J., Berwick, Z. C., Owen, M. K., Goodwill, A. G., Casalini, E. D., . . . Tune, J. D. (2013). Impaired cardiometabolic responses to glucagon-like peptide 1 in obesity and type 2 diabetes mellitus. *Basic Res Cardiol, 108*(4), 365. doi:10.1007/s00395-013-0365-x

Mogensen, U. M., Andersson, C., Fosbol, E. L., Schramm, T. K., Vaag, A., Scheller, N. M., . . . Kober, L. (2015). Metformin in combination with various insulin secretagogues in type 2 diabetes and associated risk of cardiovascular morbidity and mortality--a retrospective nationwide study. *Diabetes Res Clin Pract, 107*(1), 104-112. doi:10.1016/j.diabres.2014.09.047

Motta, A. J., Koska, J., Reaven, P., & Migrino, R. Q. (2012). Vascular protective effects of diabetes medications that mimic or increase glucagon-like peptide-1 activity. *Recent Pat Cardiovasc Drug Discov, 7*(1), 2-9.

Ogawa, S., Ishiki, M., Nako, K., Okamura, M., Senda, M., Mori, T., & Ito, S. (2011). Sitagliptin, a dipeptidyl peptidase-4 inhibitor, decreases systolic blood pressure in Japanese hypertensive patients with type 2 diabetes. *Tohoku J Exp Med, 223*(2), 133-135.

Poudyal, H. (2016). Mechanisms for the cardiovascular effects of glucagon-like peptide-1. *Acta Physiol (Oxf), 216*(3), 277-313. doi:10.1111/apha.12604

Rao, A. D., Kuhadiya, N., Reynolds, K., & Fonseca, V. A. (2008). Is the combination of sulfonylureas and metformin associated with an increased risk of cardiovascular disease or all-cause mortality?: a meta-analysis of observational studies. *Diabetes Care, 31*(8), 1672-1678. doi:10.2337/dc08-0167

Robinson, L. E., Holt, T. A., Rees, K., Randeva, H. S., & O'Hare, J. P. (2013). Effects of exenatide and liraglutide on heart rate, blood pressure and body weight: systematic review and meta-analysis. *BMJ Open, 3*(1). doi:10.1136/bmjopen-2012-001986

Rosenstock, J., Marx, N., Neubacher, D., Seck, T., Patel, S., Woerle, H. J., & Johansen, O. E. (2015). Cardiovascular safety of linagliptin in type 2 diabetes: a comprehensive patient-level pooled analysis of prospectively adjudicated cardiovascular events. *Cardiovasc Diabetol, 14*, 57. doi:10.1186/s12933-015-0215-2

Salheen, S. M., Panchapakesan, U., Pollock, C. A., & Woodman, O. L. (2015). The DPP-4 inhibitor linagliptin and the GLP-1 receptor agonist exendin-4 improve endothelium-dependent relaxation of rat mesenteric arteries in the presence of high glucose. *Pharmacol Res, 94*, 26-33. doi:10.1016/j.phrs.2015.02.003

Salling, H. K., Dohler, K. D., Engstrom, T., & Treiman, M. (2012). Postconditioning with curaglutide, a novel GLP-1 analog, protects against heart ischemia-reperfusion injury in an isolated rat heart. *Regul Pept, 178*(1-3), 51-55. doi:10.1016/j.regpep.2012.06.007

Saraiva, F. K., & Sposito, A. C. (2014). Cardiovascular effects of glucagon-like peptide 1 (GLP-1) receptor agonists. *Cardiovasc Diabetol, 13*, 142. doi:10.1186/s12933-014-0142-7

Sauve, M., Ban, K., Momen, M. A., Zhou, Y. Q., Henkelman, R. M., Husain, M., & Drucker, D. J. (2010). Genetic deletion or pharmacological inhibition of dipeptidyl peptidase-4 improves cardiovascular outcomes after myocardial infarction in mice. *Diabetes, 59*(4), 1063-1073. doi:10.2337/db09-0955

Savarese, G., Perrone-Filardi, P., D'Amore, C., Vitale, C., Trimarco, B., Pani, L., & Rosano, G. M. (2015). Cardiovascular effects of dipeptidyl peptidase-4 inhibitors in diabetic patients: A meta-analysis. *Int J Cardiol, 181*, 239-244. doi:10.1016/j.ijcard.2014.12.017

Scheen, A. J. (2012). A review of gliptins in 2011. *Expert Opin Pharmacother, 13*(1), 81-99. doi:10.1517/14656566.2012.642866

Scheen, A. J. (2013). Cardiovascular effects of gliptins. *Nat Rev Cardiol, 10*(2), 73-84. doi:10.1038/nrcardio.2012.183

Scheen, A. J. (2015). A review of gliptins for 2014. *Expert Opin Pharmacother, 16*(1), 43-62. doi:10.1517/14656566.2015.978289

Schisano, B., Harte, A. L., Lois, K., Saravanan, P., Al-Daghri, N., Al-Attas, O., . . . Tripathi, G. (2012). GLP-1 analogue, Liraglutide protects human umbilical vein endothelial cells against high glucose induced endoplasmic reticulum stress. *Regul Pept, 174*(1-3), 46-52. doi:10.1016/j.regpep.2011.11.008

Scirica, B. M., Bhatt, D. L., Braunwald, E., Steg, P. G., Davidson, J., Hirshberg, B., . . . Investigators. (2013). Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. *N Engl J Med, 369*(14), 1317-1326. doi:10.1056/NEJMoa1307684

Scirica, B. M., Braunwald, E., Raz, I., Cavender, M. A., Morrow, D. A., Jarolim, P., . . . Investigators. (2015). Heart Failure, Saxagliptin, and Diabetes Mellitus: Observations from the SAVOR-TIMI 53 Randomized Trial. *Circulation, 132*(15), e198. doi:10.1161/CIR.0000000000000330

Scott, L. J. (2017). Sitagliptin: A Review in Type 2 Diabetes. *Drugs, 77*(2), 209-224. doi:10.1007/s40265-016-0686-9

Shah, Z., Pineda, C., Kampfrath, T., Maiseyeu, A., Ying, Z., Racoma, I., . . . Rajagopalan, S. (2011). Acute DPP-4 inhibition modulates vascular tone through GLP-1 independent pathways. *Vascul Pharmacol, 55*(1-3), 2-9. doi:10.1016/j.vph.2011.03.001

Simo, R., & Hernandez, C. (2016). New Treatments for Type 2 Diabetes Mellitus and Cardiovascular Disease. The Revolution Has Begun. *Rev Esp Cardiol (Engl Ed), 69*(11), 1005-1007. doi:10.1016/j.rec.2016.07.009

Skov, J., Holst, J. J., Gotze, J. P., Frokiaer, J., & Christiansen, J. S. (2014). Glucagon-like peptide-1: effect on pro-atrial natriuretic peptide in healthy males. *Endocr Connect, 3*(1), 11-16. doi:10.1530/EC-13-0087

Ta, N. N., Schuyler, C. A., Li, Y., Lopes-Virella, M. F., & Huang, Y. (2011). DPP-4 (CD26) inhibitor alogliptin inhibits atherosclerosis in diabetic apolipoprotein E-deficient mice. *J Cardiovasc Pharmacol, 58*(2), 157-166. doi:10.1097/FJC.0b013e31821e5626

Takai, S., Sakonjo, H., & Jin, D. (2014). Significance of vascular dipeptidyl peptidase-4 inhibition on vascular protection in Zucker diabetic fatty rats. *J Pharmacol Sci, 125*(4), 386-393.

Terasaki, M., Nagashima, M., Nohtomi, K., Kohashi, K., Tomoyasu, M., Sinmura, K., . . . Hirano, T. (2013). Preventive effect of dipeptidyl peptidase-4 inhibitor on atherosclerosis is mainly attributable to incretin's actions in nondiabetic and diabetic apolipoprotein E-null mice. *PLoS One, 8*(8), e70933. doi:10.1371/journal.pone.0070933

Turner, R. C., Cull, C. A., Frighi, V., & Holman, R. R. (1999). Glycemic control with diet, sulfonylurea, metformin, or insulin in patients with type 2 diabetes mellitus: progressive requirement for multiple therapies (UKPDS 49). UK Prospective Diabetes Study (UKPDS) Group. *JAMA, 281*(21), 2005-2012.

Vila Petroff, M. G., Egan, J. M., Wang, X., & Sollott, S. J. (2001). Glucagon-like peptide-1 increases cAMP but fails to augment contraction in adult rat cardiac myocytes. *Circ Res, 89*(5), 445-452.

Wagstaff, A. J., & Goa, K. L. (2002). Rosiglitazone: a review of its use in the management of type 2 diabetes mellitus. *Drugs, 62*(12), 1805-1837.

Waldrop, G., Zhong, J., Peters, M., & Rajagopalan, S. (2016). Incretin-Based Therapy for Diabetes: What a Cardiologist Needs to Know. *J Am Coll Cardiol, 67*(12), 1488-1496. doi:10.1016/j.jacc.2015.12.058

Wang, D., Luo, P., Wang, Y., Li, W., Wang, C., Sun, D., . . . Cao, F. (2013). Glucagon-like peptide-1 protects against cardiac microvascular injury in diabetes via a cAMP/PKA/Rho-dependent mechanism. *Diabetes, 62*(5), 1697-1708. doi:10.2337/db12-1025

Wang, R., Lu, L., Guo, Y., Lin, F., Chen, H., Chen, W., & Chen, M. (2015). 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, 66*(2), 135-140. doi:10.1097/FJC.0000000000000255

White, W. B., Cannon, C. P., Heller, S. R., Nissen, S. E., Bergenstal, R. M., Bakris, G. L., . . . Investigators, E. (2013). Alogliptin after acute coronary syndrome in patients with type 2 diabetes. *N Engl J Med, 369*(14), 1327-1335. doi:10.1056/NEJMoa1305889

Widlansky, M. E., Puppala, V. K., Suboc, T. M., Malik, M., Branum, A., Signorelli, K., . . . Tyagi, S. (2017). Impact of DPP-4 inhibition on acute and chronic endothelial function in humans with type 2 diabetes on background metformin therapy. *Vasc Med*, 1358863X16681486. doi:10.1177/1358863X16681486

Willemen, M. J., Mantel-Teeuwisse, A. K., Straus, S. M., Meyboom, R. H., Egberts, T. C., & Leufkens, H. G. (2011). Use of dipeptidyl peptidase-4 inhibitors and the reporting of infections: a disproportionality analysis in the World Health Organization VigiBase. *Diabetes Care, 34*(2), 369-374. doi:10.2337/dc10-1771

Wu, S., Hopper, I., Skiba, M., & Krum, H. (2014). Dipeptidyl peptidase-4 inhibitors and cardiovascular outcomes: meta-analysis of randomized clinical trials with 55,141 participants. *Cardiovasc Ther, 32*(4), 147-158. doi:10.1111/1755-5922.12075

Xiao, Y. F., Nikolskaya, A., Jaye, D. A., & Sigg, D. C. (2011). Glucagon-like peptide-1 enhances cardiac L-type Ca2+ currents via activation of the cAMP-dependent protein kinase A pathway. *Cardiovasc Diabetol, 10*, 6. doi:10.1186/1475-2840-10-6

Yamamoto, H., Lee, C. E., Marcus, J. N., Williams, T. D., Overton, J. M., Lopez, M. E., . . . Elmquist, J. K. (2002). Glucagon-like peptide-1 receptor stimulation increases blood pressure and heart rate and activates autonomic regulatory neurons. *J Clin Invest, 110*(1), 43-52. doi:10.1172/JCI15595