

Glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1: Incretin actions beyond the pancreas

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ABSTRACT

Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are the two primary incretin hormones secreted from the intestine on ingestion of various nutrients to stimulate insulin secretion from pancreatic β -cells glucose-dependently. GIP and GLP-1 undergo degradation by dipeptidyl peptidase-4 (DPP-4), and rapidly lose their biological activities. The actions of GIP and GLP-1 are mediated by their specific receptors, the GIP receptor (GIPR) and the GLP-1 receptor (GLP-1R), which are expressed in pancreatic β -cells, as well as in various tissues and organs. A series of investigations using mice lacking GIPR and/or GLP-1R, as well as mice lacking DPP-4, showed involvement of GIP and GLP-1 in divergent biological activities, some of which could have implications for preventing diabetes-related microvascular complications (e.g., retinopathy, nephropathy and neuropathy) and macrovascular complications (e.g., coronary artery disease, peripheral artery disease and cerebrovascular disease), as well as diabetes-related comorbidity (e.g., obesity, non-alcoholic fatty liver disease, bone fracture and cognitive dysfunction). Furthermore, recent studies using incretin-based drugs, such as GLP-1 receptor agonists, which stably activate GLP-1R signaling, and DPP-4 inhibitors, which enhance both GLP-1R and GIPR signaling, showed that GLP-1 and GIP exert effects possibly linked to prevention or treatment of diabetes-related complications and comorbidities independently of hyperglycemia. We review recent findings on the extrapancreatic effects of GIP and GLP-1 on the heart, brain, kidney, eye and nerves, as well as in the liver, fat and several organs from the perspective of diabetes-related complications and comorbidities. (*J Diabetes Invest*, doi: 10.1111/jdi.12065, 2013)

KEY WORDS: Diabetic complication, Glucose-dependent insulinotropic polypeptide, Glucagon-like peptide-1

INTRODUCTION

The history of incretins dates back as early as the year 1904, when Moore, who was inspired by the discovery of secretin by Bayliss and Starling¹, hypothesized that gut extracts contain a hormone that regulates the endocrine pancreas. Based on his hypothesis, Moore showed that administration of gut extracts reduced urine sugars in diabetic patients, presumably through stimulation of the endocrine pancreas². In 1929, La Barre purified the glucose-lowering element from gut extracts, and named it incretin³. To date, gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are recognized as the two primary incretin hormones secreted from the gut after ingestion of glucose or various nutrients to stimulate insulin secretion from pancreatic β -cells glucose-dependently⁴⁻⁷. GIP and GLP-1 undergo rapid inactivation catalyzed by dipeptidyl peptidase-4 (DPP-4), and their biological activity is drastically reduced. GIP and GLP-1 exert their effects by binding to their specific receptors, the GIP receptor (GIPR) and the GLP-1 receptor (GLP-1R), which belong to the G-protein coupled receptor family.

Receptor binding activates and increases the level of intracellular cyclic adenosine monophosphate in pancreatic β -cells, thereby stimulating insulin secretion glucose-dependently. Genetic ablation of GIPR and GLP-1R separately or simultaneously in mice showed their critical roles in the entero-insular axis, and confirmed that GIP and GLP-1 act as incretins⁸⁻¹⁰. In addition to their insulinotropic actions, a series of investigations using mice lacking GIPR and/or GLP-1R, as well as mice lacking DPP-4, showed the involvement of GIP and GLP-1 in various biological effects (Figure 1), some of which could have implications for preventing or treating diabetic complications independently of hyperglycemia.

Incretin-based drugs are now being used to achieve better glycemic control in patients with type 2 diabetes worldwide¹¹⁻¹⁴. To date, two incretin-based drugs are clinically available: (i) GLP-1 receptor agonists (GLP-1RA) that increase resistance to DPP-4 degradation to enable strong and steady activation of GLP-1R; and (ii) DPP-4 inhibitors (DPP-4i) that inhibit DPP-4-dependent inactivation of GIP and GLP-1, thereby enhancing their various biological actions. A series of clinical trials of GLP-1RA and DPP-4i showed that they significantly lower glycated hemoglobin without serious hypoglycemia and bodyweight gain¹⁵⁻¹⁸. It was noticed that incretin-based drugs are more effective in Asians, likely a result of amelioration of defective early

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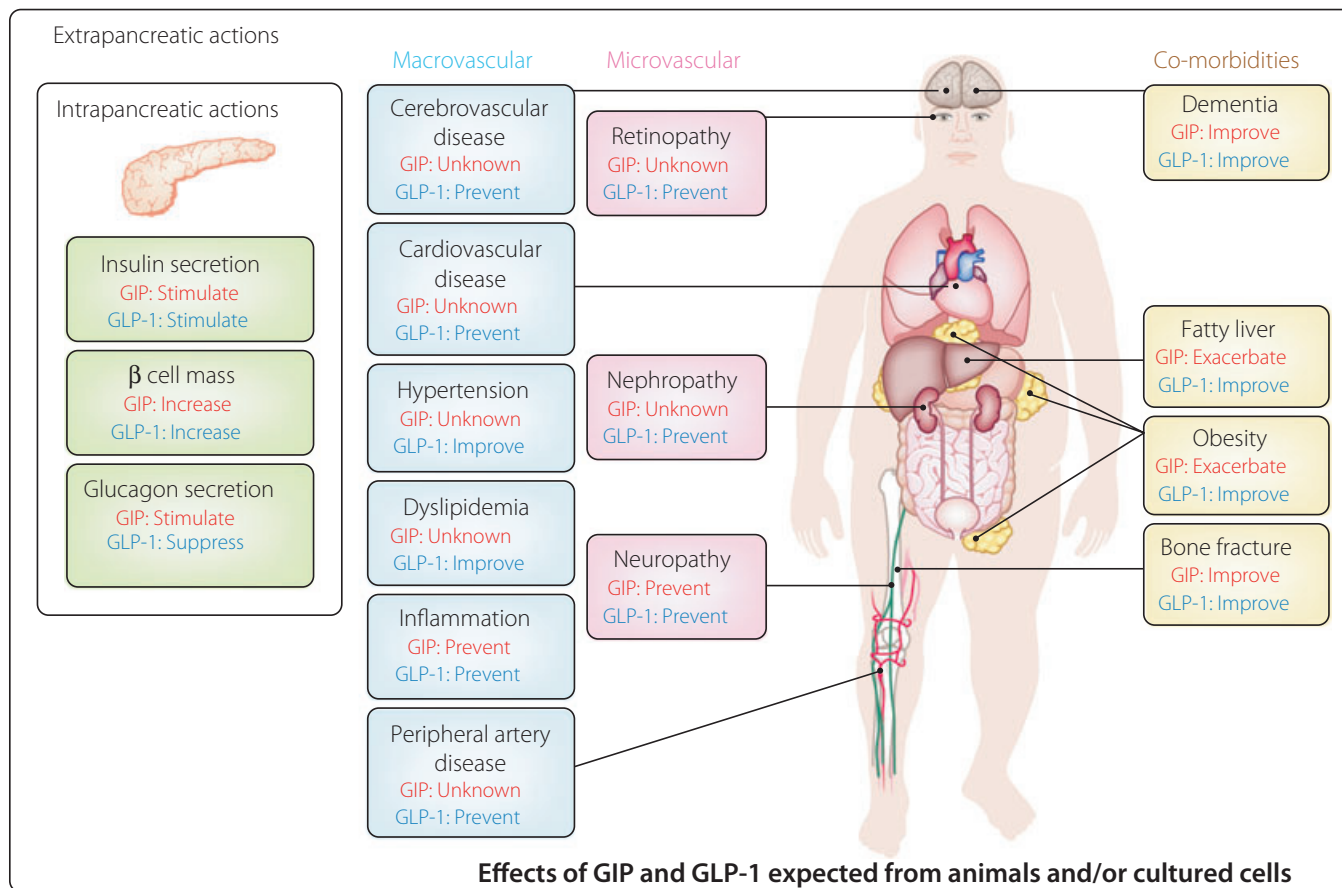


Figure 1 | Extraprocreatic actions of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), and implications for diabetes-related complications and comorbidities. GIP and GLP-1 exert various intra- and extraprocreatic actions suggested from animal studies, some of which might be beneficial for prevention and treatment of diabetes-related complications and comorbidities shown independently of glycemic control. Note that beneficial and adverse effects of GIP and GLP-1 listed largely await confirmation in patients with diabetes.

phase insulin secretion, which is a characteristic feature of type 2 diabetes in Asians^{4,19}. Recent meta-analyses of DPP-4i therapy support this notion^{20,21}. Large prospective clinical trials are ongoing to ascertain the effects of incretin-based drugs on diabetes-related complications of patients with type 2 diabetes, but the results are not yet available (Table 1). Thus, our knowledge today is still largely restricted to results obtained from experimental animals and cultured cells.

Diabetes-related vascular complications are the major causes of morbidity and mortality in patients with diabetes. Microvascular complications include retinopathy, nephropathy and neuropathy, which are the leading causes of blindness, renal failure and nerve injuries associated with foot ulcers and amputations, respectively. Macrovascular complications involve atherosclerosis-related diseases, such as coronary artery disease (CAD), peripheral artery disease (PAD), cerebrovascular disease (CVD) and possible cognitive dysfunction. Classical clinical trials, such as the Diabetic Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS),

clearly showed that intensive antidiabetic treatment can ameliorate hyperglycemia and reduce the progression of microvascular complications^{22,23}; the follow up of DCCT and UKPDS also showed that such intensive treatment can suppress the incidence of macrovascular complications^{24,25}. These findings show that hyperglycemia is a critical cause of diabetes-related vascular complications, and provide a basis to search for strategies to obtain better glycemic control. In addition, various studies have clarified mechanisms underlying the pathogenesis of diabetic vascular complications including: (i) the polyol pathway; (ii) the protein kinase C (PKC) pathway; (iii) oxidative stress; (iv) advanced glycation end-products (AGEs) pathway; and (v) the hexosamine pathway²⁶. All of these findings are suggestive for the development of agents to prevent diabetes-related vascular complications independently of glycemic conditions.

In the present review, we highlight recent findings on the extraprocreatic effects of GIP and GLP-1 in various tissues and organs that might be relevant to the prevention and management of diabetes-related complications.

Table 1 | Cardiovascular outcomes trials with selected incretin-based drugs.

Trial name (ClinicalTrials.gov ID)	Agent	Description
CAROLINA (NCT01243424)	Linagliptin	Comparator: Glimepiride (superiority if non-inferiority met) Period: October 2010–September 2018 Time to first occurrence of major CV events (Approximately 6,000 high risk type 2 diabetes; >5 years), multinational
EXAMINE (NCT00968708)	Alogliptin	Comparator: Placebo (non-inferiority design) Period: September 2009–December 2013 Time to first occurrence of primary major adverse cardiac events (Approximately 5,400 type 2 diabetes with ACS; 5 years), multinational
SAVOR-TIMI53 (NCT01107886)	Saxagliptin	Comparator: Placebo (superiority design) Period: May 2010–April 2013 To evaluate the effect on major CV events (Approximately 16,500 high-CV-risk type 2 diabetes; 4 years), multinational
TECOS (NCT00790205)	Sitagliptin	Comparator: Placebo (non-inferiority design) Period: December 2008–December 2014 Time to first occurrence of composite CV outcome (>14,000 type 2 diabetes; >4 years), multinational
ELIXA (NCT01147250)	Lixisenatide	Comparator: Placebo (superiority design) Period: June 2010–May 2013 To evaluate the effect of lixisenatide on CV morbidity and mortality (Approximately 6,000 type 2 diabetes with ACS; until occurrence of Approximately 840 primary events), multinational
EXSCAL (NCT01144338)	Exenatide Once weekly	Comparator: Placebo (superiority design) Period: June 2010–March 2017 To evaluate the effect of exenatide once weekly on major CV events (>9,000 type 2 diabetes; >5 years), multinational
LEADER (NCT01179048)	Liraglutide	Comparator: Placebo (superiority design) Period: August 2010–January 2016 To evaluate the effect on CV outcomes (Approximately 9,000 high-CV-risk type 2 diabetes; 5 years), multinational
REWIND (NCT01394952)	Dulaglutide	Comparator: Placebo (superiority design) Period: July 2011–April 2019 Time to first occurrence of major CV events (Approximately 9,600 type 2 diabetes with established CV risk; approximately 6.5 years), multinational

ACS, acute coronary syndrome; CV, cardiovascular.

DIABETES-RELATED MACROVASCULAR COMPLICATIONS

Several studies showing the effects of GLP-1 and GIP on macrovascular function have recently been published. We summarize the effects of GLP-1 and GIP that might be of significance for treatment of macrovascular complications, such as CAD, PAD and CVD, in patients with diabetes.

Effects on Atherosclerosis

Atherosclerosis constitutes the underlying pathological lesion in the clinical entities CAD, CVD and PAD. The inflammatory responses induced by adhesion of monocytes to the vascular wall have been known to play important roles in the early stages of the development of atherosclerosis. GLP-1 was shown to significantly inhibit macrophage infiltration and atherosclerosis development in normal and diabetic apolipoprotein E-deficient mice (Figure 2)^{27–29}, whereas a recent study using the long-acting GLP-1RA tasoglutide failed to reproduce attenuation of

atherosclerosis by GLP-1R activation³⁰. Exendin-4 significantly inhibited monocyte adhesion to the vascular endothelial cells by reducing production of intercellular adhesion molecule (ICAM)-1²⁹. Liraglutide also attenuated the expressions of vascular adhesion molecules (VAM) and ICAM-1 in human vascular endothelial cells, thereby suppressing development of atherosclerosis^{31,32}. In patients with diabetes, it has been shown that exenatide suppressed markers for inflammation, high sensitive C-reactive protein and monocyte chemo-attractant protein-1 (MCP-1), oxidative stress, and prostaglandin F₂ α ³³, and that exenatide improved induced endothelial dysfunction^{34,35}.

Although GLP-1R expression in mouse macrophages is still controversial³⁰, it has been shown that exendin-4 somehow inhibits lipopolysaccharide (LPS)-induced production of the inflammatory cytokine tumor necrosis factor (TNF)- α and MCP-1 from isolated mouse macrophages²⁹. Exendin-4 suppressed the nuclear translocation of p65, a component of nuclear factor (NF)- κ B, and this effect was reversed by both

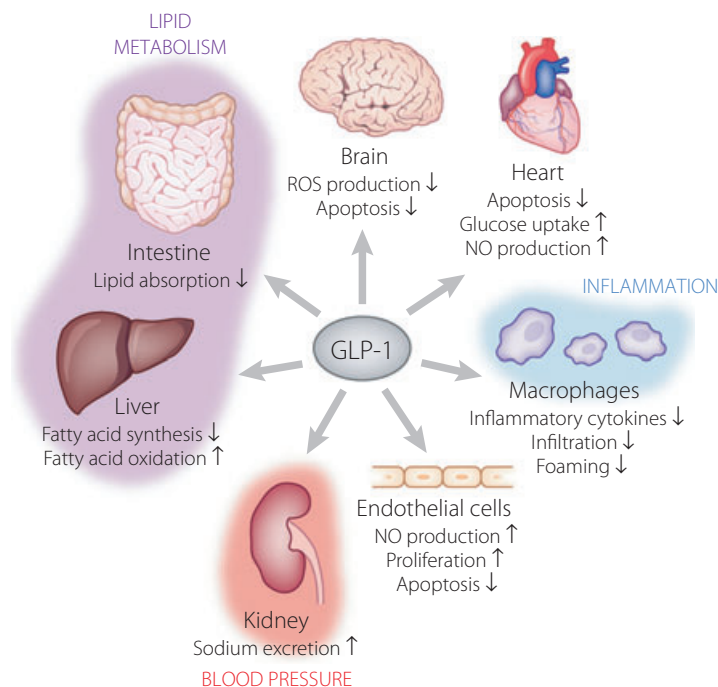


Figure 2 | Glucagon-like peptide-1 (GLP-1) prevents macrovascular complications through multiple mechanisms. GLP-1 prevents macrovascular complications, such as coronary artery disease, cerebrovascular disease and peripheral artery disease, through direct actions on the brain, heart and vascular endothelial cells. GLP-1 also indirectly exerts beneficial effects through regulation of lipid metabolism, blood pressure and inflammation. NO, nitric oxide; ROS, reactive oxygen species.

MDL-12330A, a cyclic adenosine monophosphate (cAMP) inhibitor, and PKI14-22, a protein kinase A (PKA)-specific inhibitor. Therefore, it was suggested that these actions of exendin-4 are likely to be mediated through a GLP-1R/PKA pathway in mouse macrophages²⁹. It also has been shown that GLP-1 activates human macrophages through signal transducers and activator of transcription (STAT3) activation³⁶.

In addition to its effects on macrophages, it has been shown that GLP-1 exerts direct effects on vascular endothelial cells (Figure 2). GLP-1R activation directly activated endothelial nitric oxide synthase (eNOS) in human umbilical vein endothelial cells (HUVECs)³⁷ and the aortic endothelium³¹. GLP-1 increased production of nitric oxide (NO), resulting in an increase of the microvascular blood flow in the blood³⁸. It was reported that NO production was mediated by the poly(adenosine diphosphate-ribose) polymerase pathway³⁹. GLP-1 also promoted proliferation and differentiation of endothelial progenitor cells by upregulating vascular endothelial growth factor (VEGF) expression⁴⁰. Incubation of human coronary artery endothelial cells (HCAECs) with exendin-4 resulted in a dose-dependent increase in DNA synthesis and an increased cell number associated with enhanced eNOS and v-akt murine thymoma viral oncogene homolog 1 (Akt1) activation that were abolished by a GLP-1R antagonist⁴¹; Exendin-4 directly improves endothelial dysfunction in isolated aortas⁴². Liraglutide also inhibited the NF- κ B pathway and suppressed apoptosis of HUVECs⁴³. There is a report that GLP-1 also

inhibited AGE-induced apoptosis in HUVECs⁴⁴. Taken together, it appears that GLP-1 can exert anti-atherogenic effects through various mechanisms.

GIP was also shown to significantly inhibit macrophage infiltration and atherosclerosis development in normal and diabetic apolipoprotein E-deficient mice^{27,28}. It was recently found that GIP inhibits AGEs-enhanced production of reactive oxygen species (ROS), and expression of VAM-1 and PAI-1 through the GIPR/Epac pathway⁴⁵. These findings show that GLP-1 and GIP can exert anti-atherosclerosis effects, and might have implications in preventing macrovascular complications.

Consistent with the anti-atherosclerotic effects of GLP-1 and GIP, DPP-4i des-fluor-sitagliptin was also shown to inhibit the production of inflammatory cytokines, such as TNF- α , interleukin (IL)-6, IL-1 β and MCP-1, as well as that of VAM-1 and ICAM-1, and to increase endothelial NO production and suppress the NF- κ B pathway in experimental animals⁴⁶. Furthermore, they showed that DPP-4 inhibition increases circulating endothelial precursor cells (EPCs), which exert vasoprotective effects⁴⁶. Similar observations were made with various DPP-4i including sitagliptin⁴⁷, alogliptin^{47,48} and vildagliptin^{49,50}. Such anti-atherosclerotic effects were also observed in type 2 diabetic patients treated with sitagliptin^{51–53}.

Effect on Cardiac Function

Studies in experimental animals have shown beneficial roles of GLP-1 or GLP-1RA in CAD (Figure 2). In pigs, exendin-4

administration significantly reduced infarct size and improved wall motion⁵⁴. In mice, liraglutide administration showed similar cardioprotective effects with activation of cardioprotective genes⁵⁵. In rabbits, administration of GLP-1 fused to human transferrin significantly reduced infarct size and improved the wall motion and ejection fraction after myocardial ischemia/reperfusion injury⁵⁶. Like GLP-1RA, native GLP-1 also showed a cardioprotective function in rats with myocardial ischemia/reperfusion injury, partly by suppressing activation of peripheral neutrophil⁵⁷. Another study on dogs with myocardial ischemia suggested that GLP-1 exerted a cardioprotective role by augmenting glucose uptake⁵⁸. This is consistent with a recent study showing that the long-acting GLP-1RA albiglutide enhanced myocardial glucose uptake and promoted a shift toward a more energetically favorable substrate metabolism by increasing both glucose and lactate oxidation, thereby reducing infarct size and improving cardiac function⁵⁹. Despite recent debates on GLP-1R expression in mouse hearts, the earlier study showed that GLP-1R activation increases cAMP production and suppresses caspase-3 activation in cardiomyocytes, thereby preventing apoptosis of cardiomyocytes⁵⁵. Another study showed that GLP-1R activation increases cAMP and phosphorylation of Akt and extracellular signal-regulated kinase (ERK), regulators of growth and glucose metabolism in cardiomyocytes⁶⁰. Importantly, it was shown that although GLP-1 increased cAMP levels in cardiomyocytes, the increased cAMP did not correlate with the intracellular calcium concentration and subsequent cardiomyocyte contractility⁶¹. It is interesting to note that GLP-1 was previously shown to exert cardioprotective effects mediated through both GLP-1R-dependent and GLP-1R-independent mechanisms⁶². It was suggested that GLP-1R-independent cardioprotective actions of GLP-1 are mediated by GLP-1(9-36)amide, the primary GLP-1 metabolite *in vivo*, because GLP-1(9-36)amide exerts cardioprotective actions in GLP-1R-deficient mice⁶⁰. However, the molecular mechanisms underlying the GLP-1R-independent cardioprotective actions of GLP-1(9-36)amide are largely unknown. Little is known of the effects of GIP on the heart.

In a clinical study, it was reported that GLP-1 significantly improved left ventricle ejection fraction, global wall motion indices, and regional wall motion indices in patients with acute myocardial infarction and severe systolic dysfunction⁶³. More recently, it was reported that GLP-1 infusion protects the heart from ischemic left ventricle dysfunction induced by dobutamine stress in CAD patients⁶⁴. Furthermore, it was shown that xenatide reduces reperfusion injury and final infarct size in patients with ST-segment elevated myocardial infarction^{65,66}.

Studies in experimental animals showed that DPP-4 inhibition also results in similar cardioprotective effects⁶⁷⁻⁷². It has been shown that the DPP-4i sitagliptin increased the intracellular cAMP and PKA activity, and that H-89, a potent selective PKA inhibitor, completely blocked the effect of sitagliptin in reducing the size of myocardial infarct, suggesting involvement of the GLP-1R/cAMP/PKA pathway⁷⁰. However, a recent study

also suggested that the cardioprotective effects of DPP-4i are mediated not only by GLP-1, but also by other bioactive peptides, such as stromal-derived factor-1 α ^{51,73,74}.

It was also suggested that GLP-1R activation might exert beneficial effects in heart failure. In a dog model of pacing-induced heart failure, GLP-1 increased glucose uptake, thereby increasing left ventricular function⁷⁵. In the spontaneously hypertensive and heart failure-prone rat, GLP-1 infusion improved survival and preserved left ventricular function with reduced apoptosis of cardiomyocytes⁷⁶. In a rat model of chronic heart failure with permanent occlusion of the left anterior descending artery, infusion of GLP-1 or GLP-1RA enhanced left ventricular function, reducing left ventricular remodeling and improving survival⁷⁷. In humans, infusion of GLP-1 or GLP-1RA in patients with heart failure improved left ventricular ejection fraction^{78,79}. Furthermore, it has been shown that DPP-4i improved left ventricular function in experimental models of heart failure^{71,80}. Although further studies of the underlying mechanisms are required, these results together strongly suggest clinical implications for GLP-1RA in treatment of heart failure.

Effect on Cerebrovascular Function

Neuroprotective effects of GLP-1RA after cerebral ischemia in non-diabetic and diabetic animals have been described (Figure 2)⁸¹⁻⁸³. Intravenous injection of exendin-4 after cerebral ischemia reduced the infarct size and neurological deficits induced by reperfusion after occlusion of the middle cerebral artery in a mouse model of acute cerebral infarction^{81,83}. Exendin-4 also attenuated the oxidative stress and reduced neuronal cell death after reperfusion in this focal ischemia model. As exendin-4 injection has been associated with increased intracellular cAMP levels⁸¹, this compound likely exerts its neuroprotective effect through a cAMP activation pathway. It therefore appears that GLP-1RA might be potentially useful in the treatment of cerebral infarction. No study has been carried out on the neuroprotective effects of GIP after cerebral ischemia, although it has been shown that GIPR-deficient mice showed impaired learning, synaptic plasticity and neurogenesis⁸⁴, and that GIP and GIPR agonist enhance long-term potentiation (LTP) and neurogenesis^{85,86}. Consistent with the neuroprotective effects of GLP-1RA after cerebral ischemia, the DPP-4i linagliptin was found to reduce ischemic brain damage in diabetic mice⁸⁷. In the same study, sulfonylurea glibenclamide lowered glucose levels, but did not show similar neuroprotective effects, suggesting that the neuroprotective effects are independent of glycemic control and presumably mediated by GLP-1⁸⁷.

Effect on Blood Pressure

Hypertension plays a critical role in the development of macrovascular complications. Chronic infusion of GLP-1 reduced the incidence of hypertension, and prevented cardiac hypertrophy and fibrosis in salt-sensitive Dahl rats⁸⁸. In this model of hypertension, GLP-1 also reduced urinary albumin excretion, increased urinary sodium excretion and improved

histopathological abnormalities, such as glomerulosclerosis and tubular necrosis (Figure 2)⁸⁸. In salt-sensitive obese *db/db* mice and angiotensin II-infused C57BL/6J mice, exendin-4 prevented the onset of hypertension and increased the urinary sodium excretion⁸⁹. Similarly, infusion of GLP-1RA AC3174 also attenuated hypertension and the histopathological changes associated with the renal dysfunction in the Dahl rats⁹⁰. Administration of DPP-4i sitagliptin also reduced blood pressure in spontaneously hypertensive rats by decreasing expression of Na⁺/H⁺ exchanger isoform 3 in microvilli membranes of the proximal renal tubule, thereby increasing the urinary sodium excretion and the urinary volume, and reducing blood pressure⁹¹. Furthermore, GLP-1RA and DPP-4i also ameliorates endothelial dysfunction⁹².

Antihypertensive effects of GLP-1 have consistently been shown in several clinical trials. In the trials to evaluate efficacy and safety of GLP-1RA liraglutide carried out in type 2 diabetic patients, liraglutide administration was found to decrease systolic blood pressure by 2–6 mmHg from baseline in 26 weeks⁹³. In addition, measurement of flow-mediated vasodilatation (FMD) of the brachial artery as a measure of endothelial function in patients with type 2 diabetes after 16-week exenatide treatment showed a significantly higher value of FMD in the exenatide-treated group compared with that in the glimepiride-treated group⁹⁴. This result shows that exenatide exerts vasodilatory action and might reduce blood pressure.

Increase of urinary sodium excretion and urinary volume are well-known effects of GLP-1 and GLP-1RA in rodents. In rats, intracerebroventricular injection of GLP-1 was found to exert marked natriuretic and diuretic effects mediated by GLP-1R that were blocked by treatment with GLP-1R antagonist exendin(9-39)⁹⁵. GLP-1 was expressed in porcine proximal tubular cells isolated from kidneys, and inhibited sodium reabsorption⁹⁶. It is therefore likely that the marked diuretic effect of GLP-1 is mediated by direct regulation of sodium reabsorption in kidney proximal tubules, as well as through hypothalamic GLP-1R. Clinically, the effects of GLP-1 infusion on urinary sodium excretion, urinary output and the glomerular filtration rate after an intravenous administration of salt load were investigated in obese men; GLP-1 was found to significantly enhance the urinary sodium excretion, H⁺ secretion and glomerular hyperfiltration in obese men⁹⁷.

Although no report has shown an association of GIP with blood pressure, DPP-4i sitagliptin attenuated elevation of blood pressure in spontaneously hypertensive rats^{91,92}. It also has been reported that DPP-4i reduces blood pressure^{98,99}, which supports the notion that GLP-1 regulates blood pressure in humans.

Effect on Dyslipidemia

Dyslipidemia plays a critical role in the development of macrovascular complications. It has been shown that GLP-1 ameliorates dyslipidemia in experimental animals, as well as in humans, whereas little is known on the effects of GIP on lipid metabolisms.

GLP-1 infusion reduced apolipoprotein B-48 production and triglycerides absorption (Figure 2)¹⁰⁰. These effects were reproduced in mice and hamsters infused with exendin-4, which acutely decreased postprandial serum triacylglycerol and apolipoprotein B-48 GLP-1R-dependently¹⁰¹. These effects were observed even if exendin-4 was given 1 h after fat ingestion, showing that the effects on postprandial lipid metabolism were not related to delayed gastric emptying¹⁰¹. Secretion of apolipoprotein B-48 was significantly reduced from hamster primary enterocytes treated by exendin-4¹⁰¹, suggesting that GLP-1R activation expressed on enterocytes controls secretion of chylomicron. GLP-1 controls hepatic lipid metabolism. GLP-1RA markedly reduced hepatic lipid content by suppressing genes involved in fatty acid synthesis (e.g., sterol-regulatory element binding protein-1c, fatty acid synthase and steroyl CoA desaturase-1) and enhancing expression genes regulating fatty acid oxidation (e.g., acyl-coenzyme A oxidase and carnitine palmitoyltransferase 1a)^{30,102,103}. Mechanisms regulating expression of genes involved in lipid metabolism by GLP-1RA are largely unknown, inasmuch as the presence of hepatic GLP-1R expression is still controversial. Nevertheless, both GLP-1 and GLP-1RA clearly ameliorate dyslipidemia in experimental animals, suggesting clinical implications in patients with dyslipidemia.

GLP-1 infusion inhibited the postprandial elevation of triglycerides and free fatty acids in healthy human subjects¹⁰⁴. A single subcutaneous injection of exenatide in patients with newly diagnosed type 2 diabetes also showed marked reduction in postprandial triacylglycerol, as well as in apolipoprotein B-48¹⁰⁵. Although these effects of GLP-1 or GLP-1RA on triglycerides and free fatty acids could be partly a result of delayed gastric emptying, these results clearly show that GLP-1R activation ameliorates postprandial lipidemia. It has also been shown that DPP-4i vildagliptin and sitagliptin suppressed postprandial elevation of triglycerides and apolipoprotein B-48 in patients with type 2 diabetes^{106,107}. As DPP-4i shows little effect on gastric emptying¹⁰⁸, the effects of DPP-4i on postprandial lipidemia might be largely mediated by inhibition of intestinal lipid absorption. It is noteworthy that DPP-4i ameliorates dyslipidemia in patients with type 2 diabetes¹⁰⁹, which, although small, could also contribute to the prevention of macrovascular complications.

DIABETES-RELATED MICROVASCULAR COMPLICATIONS

GIPR and GLP-1R are expressed in the peripheral and central nervous system, the eyes, and the kidney, suggesting the possibility of some effects in these organs. We summarize the effects of GIP and GLP-1 in those organs that are likely to be of significance in the treatment of the microvascular complications (i.e., retinopathy, nephropathy and neuropathy) of diabetes.

Effect on the Peripheral Nervous System

Most recognized diabetes-related neurological complications involve the peripheral nervous system¹¹⁰. Several studies have

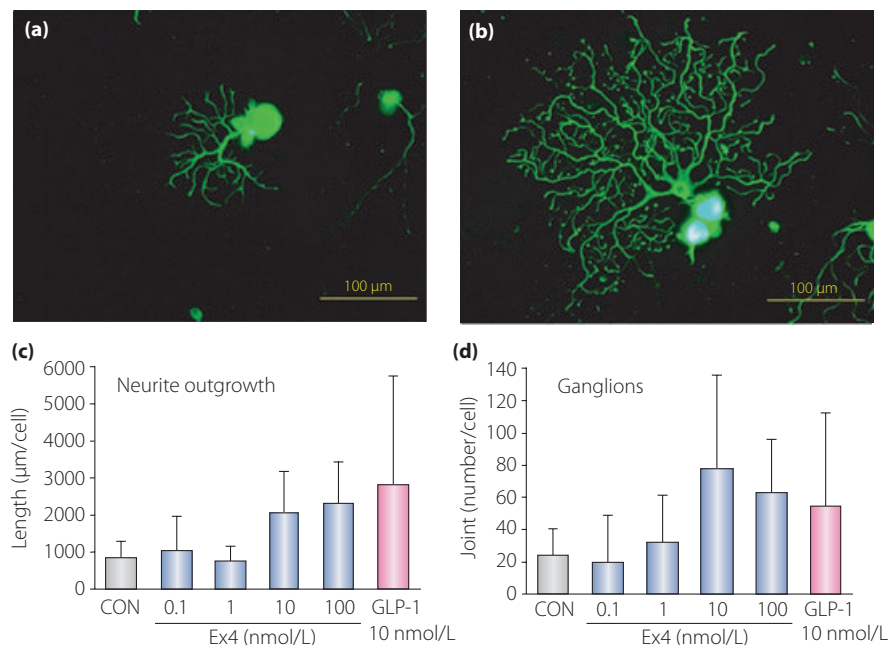


Figure 3 | Glucagon-like peptide-1 (GLP-1) receptor activation promotes neurite outgrowth of the dorsal root ganglion. Representative fluorescence micrograph of dorsal root ganglion neurons cultured in the (a) absence or (b) presence of GLP-1. GLP-1 or GLP-1 receptor agonist exendin-4 (Ex4) increased the total neurite length (c) and joint number (d) of neurites. CON, control. Reproduced from Himeno *et al.*¹¹¹, with permission from the American Diabetes Association © 2011.

shown beneficial effects of GLP-1 and GIP on the peripheral nervous system that might have therapeutic implications for the treatment of diabetic neuropathy.

GLP-1R has been found to be expressed in the lumbar dorsal root ganglion neurons (DRG) of C57BL/6/J mice¹¹¹. GLP-1 and GLP-1RA exendin-4 significantly promoted the neurite outgrowth of DRG neurons (Figure 3). Exendin-4 attenuated the hypoalgesia, and delayed motor and sensory nerve conduction velocity in diabetic mice¹¹². In the same study, exendin-4 also ameliorated the decrease in intra-epidermal nerve fiber densities in the sole skins of the diabetic mice. These findings suggest that exendin-4 might ameliorate the severity of diabetic polyneuropathy through exerting direct actions on the DRG neurons and their axons. GLP-1R was also found to be expressed in the sciatic nerve¹¹². GLP-1RA significantly increased the phosphorylated ERK 1/2 levels in the sciatic nerves of diabetic rats, showing GLP-1R activity in this diabetic tissue. Exendin-4 exerted no effect on the blood sugar or insulin levels in diabetic mice, and also had no effect on paw thermal response latencies in these mice, but attenuated the reductions of motor nerve conduction velocity and paw intra-epidermal fiber density seen in diabetic mice. Thus, GLP-1R-mediated ERK-signaling in the sciatic nerve of diabetic rodents might protect the large-motor fiber functions and small C fiber structure by a mechanism independent of glycemic control¹¹³. GLP-1R has been found to be expressed in the skin. Exendin-4 treatment reduced the increase in the current perception threshold seen in diabetic

rats. The decrease in the size of myelinated fibers or in the axon/fiber area ratio in the sciatic nerve and loss of the intra-epidermal nerve fibers in the skin of diabetic rats were also ameliorated by exendin-4 treatment. Thus, exendin-4 might prevent the peripheral nerve degeneration seen in diabetic rats, suggesting that GLP-1RA might be useful in the treatment of peripheral neuropathy¹¹². Pyridoxine-induced peripheral neuropathy is characterized by sensory nerve conduction deficits associated with disturbances of the nerve fiber geometry and axonal atrophy. In an evaluation carried out using behavioral and morphometric techniques, GLP-1 and GLP-1RA exendin-4 were found to improve pyridoxine-induced sensory peripheral neuropathy in rats¹¹⁴. Based on these findings, it has been suggested that GLP-1RA might be useful in the treatment of diabetic neuropathy.

Expressions of GIP and GIPR were enhanced after sciatic nerve crush injury in DRG, spinal cord and nerve fragments of rats, suggesting involvement of GIP/GIPR in axonal regeneration. Indeed, GIPR-deficient mice show impaired axonal regeneration¹¹⁵. Thus, GIPR activation might have therapeutic implications for the treatment of diabetic neuropathy.

Consistent with the beneficial effects of GIP and GLP-1 on the peripheral nervous system, DPP-4i vildagliptin analog PKF275-055 partially counteracted the nerve conduction velocity deficit observed in diabetic rats¹¹⁶. Diabetic rats developed mechanical hyperalgesia and showed significantly longer thermal response latencies¹¹⁷. PKF275-055 induced recovery of the

mechanical sensitivity thresholds by approximately 50% and progressively improved the alterations in the thermal responsiveness. DPP-4i is therefore likely to have a potential therapeutic effect in the treatment of diabetic neuropathy. Vildagliptin was found to protect against nerve fiber loss in diabetic animals¹¹⁷. The decrease in intra-epidermal nerve fiber density in diabetic rats was significantly inhibited by vildagliptin treatment¹¹⁷. Based on these results, it is suggested that DPP-4i might prevent peripheral nerve degeneration in diabetic animals and might be useful in treatment of peripheral neuropathy.

Effect on the Kidney

Diabetic nephropathy is a critical diabetes-related microvascular complication that is a major cause of renal failure^{118,119}. Recently, many studies have been carried out to investigate the effects of GLP-1, GLP-1RA and DPP-4i on kidney dysfunctions, but little is known about the effect of GIP on kidney. We review the effects of GLP-1, GLP-1RA and DPP-4i on the kidney functions that might be of significance in the treatment of diabetic nephropathy.

GLP-1R messenger ribonucleic acid (mRNA) was detected in rat glomeruli and glomerular endothelial cells, as well as in human monocytes and macrophages¹²⁰. In diabetic *db/db* mice,

exendin-4 was found to reduce the urinary albumin excretion and histological parameters of glomerular injury characterized by mesangial extracellular matrix expansion and glomerulosclerosis (Figure 4)¹²¹. Exendin-4 also significantly reduced the expression of transforming growth factor (TGF)- β 1, deposition of type-IV collagen, macrophage infiltration and apoptosis in the glomeruli¹²⁰. Similarly, it also reduced the glomerular hypertrophy and mesangial matrix expansion noted in diabetic rats¹²⁰. Exendin-4 also reduced the marker levels of macrophage infiltration, plasma levels of ICAM-1, deposition of type-IV collagen, oxidative stress and NF- κ B activation in the kidney tissue¹²⁰. Furthermore, exendin-4 attenuated the production of high glucose-stimulated inflammatory cytokines, such as TNF- α and IL-1 β , in human acute monocytic leukemia cell line THP-1 cells and human glomerular endothelial cells¹²⁰. These effects were significantly blocked by the GLP-1 antagonist, exendin (9-39)¹²⁰. GLP-1RA liraglutide normalized the levels of the oxidative stress markers, 8-hydroxydeoxyguanosine and malondialdehyde, and expressions of the nicotinamide adenine dinucleotide phosphate oxidase components TGF- β and fibronectin in the renal tissues, and also decreased the urinary albumin excretion that was significantly increased in diabetic rats¹²². Native GLP-1 has been shown to suppress the AGE receptor and reduce MCP-1 expression in mesangial cells¹²³.

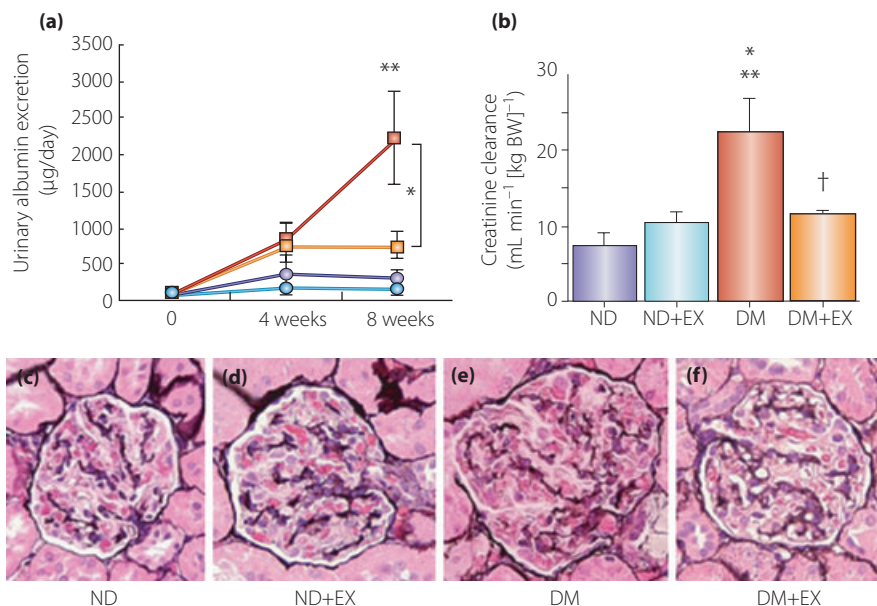


Figure 4 | Glucagon-like peptide-1 prevents kidney lesions in streptozotocin-induced diabetic rats. (a) Changes in 24-h urinary albumin excretion. Urinary albumin excretion increased gradually over 8 weeks in the diabetic group. Exendin-4 resulted in a significantly lower level of urinary albumin excretion at 8 weeks than in the untreated diabetes group. * $P < 0.05$; ** $P < 0.01$ versus non-diabetic and non-diabetic + exendin-4 groups. Blue circles, non-diabetic group (ND); light blue circles, non-diabetic + exendin-4 (ND+EX); red squares, streptozotocin-induced diabetes group (DM); yellow squares, diabetes + exendin-4 group (DM+EX). (b) Creatinine clearance. Hyperfiltration in DM was significantly decreased by DM+EX at 8 weeks. * $P < 0.05$ versus ND+EX; ** $P < 0.01$ versus ND; † $P < 0.05$ versus DM. Periodic acid-methenamine-silver (PAM) staining in (c) glomeruli of ND, (d) ND+EX, (e) DM and (f) DM+EX (magnification: $\times 200$). Mesangial matrix index, calculated by the PAM-positive area in the tuft area, was significantly increased in DM, whereas mesangial matrix expansion was significantly reduced in DM+EX. Reproduced from Kodera *et al.*¹²⁰, with permission from *Diabetologia* © 2011.

Furthermore, recent studies suggested involvement of PKA and PKC β activation in the renoprotective effects of GLP-1^{124,125}. Consistent with the renoprotective effects of GLP-1, DPP-4i vildagliptin significantly reduced proteinuria, albuminuria and the urinary albumin/creatinine ratio in diabetic rats¹²⁶. Vildagliptin also improved the creatinine clearance, and inhibited the histological changes of interstitial expansion, glomerulosclerosis and thickening of the glomerular basement membrane in a dose-dependent manner in diabetic rats. These renoprotective effects of vildagliptin were related to a reduction in the production of the inflammatory TGF- β 1. Vildagliptin also exerted renoprotective effects in rats with renal ischemia-reperfusion injury¹²⁷. Sitagliptin ameliorated renal lesions in the diabetic rats, including glomerular, tubulointerstitial and vascular lesions, accompanied by reduced lipid peroxidation¹²⁸.

Taking these results together, it is likely that GLP-1RA and DPP-4i might have potential renoprotective effects in patients with diabetic nephropathy. Indeed, it has been recently shown that exenatide reduced urinary TGF- β 1 and type-IV collagen excretion in patients with type 2 diabetes¹²⁹, and that sitagliptin reduced albuminuria in patients with type 2 diabetes¹³⁰. However, it remains to be seen if the renoprotective effects in patients depend on amelioration of glycemic control or if GLP-1 can exert direct effects on the kidney.

Effect on the Eye

Diabetic retinopathy is one of critical diabetes-related microvascular complications that occurs in approximately 60% of patients with duration of diabetes more than 20 years, and is one of the major causes of blindness¹³¹. Some studies regarding the effects of GLP-1RA and DPP-4i on the retina have been available for several years^{132–135}. In contrast, the effects of GIP on the retina are scarcely known, although it has been shown that GIP and GIPR are expressed in the rat retina^{136,137}. We summarize the effects of GLP-1, GLP-1RA and DPP-4i on the retina that have potential clinical significance in the treatment of retinopathy.

Both the mRNA and protein expressions of GLP-1R have been detected in the inner layer of the retina in rats¹³². In diabetic rats, subcutaneous injection of exendin-4 prevented the loss of the b-wave amplitude and oscillatory potentials in the retina¹³². The retinal thickness, which depends on the duration of diabetes, is reduced, and the cell counts of both the outer and inner nuclear layers are also reduced in diabetic rats; however, subcutaneous administration of exendin-4 prevented the loss of the retinal cells and maintained the normal retinal thickness. In addition, intravitreal injection of exendin-4, independent of its pancreatic effect, also prevented both the loss of the b-wave amplitude and the oscillatory potentials observed in diabetes, and also the cell loss in the outer and inner nuclear layers in the retina¹³³. The excessive retinal glutamate was significantly reduced by exendin-4 treatment in diabetic rats. Rapid removal or inactivation of glutamate is required to maintain the normal functions of the retina through the pre-

vention of glutamate-induced injury of the neurons. Consistent with such changes, retinal GLP-1R and glutamate aspartate transporter expressions were also reduced in the diabetic retina, but were upregulated in the exendin-4-treated rats¹³³. The effect of exendin-4 in improving glucose-induced retinal ganglion cell impairment has also been investigated. Exendin-4 protected retinal ganglion cells by affecting expression in proapoptotic Bax and anti-apoptotic Bcl-2, and the protective effects of exenatide were inhibited by GLP-1R antagonist, exendin(9-39)¹³⁴. Taking these results together, GLP-1R activation might well protect retinal functions in diabetic retinopathy.

In diabetic rats, DPP-4i sitagliptin reduced the intracellular accumulation of tight junction proteins, such as occludin and claudin-5, in the blood retinal barrier observed in diabetes¹³⁵. Sitagliptin also decreased the nitrosative stress, inflammatory IL-1 β production and cell death by apoptosis in diabetic retinas¹³⁵. Sitagliptin recovered the number of circulating CD34+ cells to the control level in diabetic rats and increased the adhesion ability of endothelial progenitor cells to the retinal vessels¹³⁵. These results suggest that DPP-4i might exert beneficial effects on the integrity of the blood–retinal barrier in diabetic rat retinas through prevention of nitrosative stress, inflammatory responses and apoptosis of the retinal cells, although it needs to be tested whether these beneficial effects are mediated by GLP-1.

DIABETES-RELATED COMORBIDITIES

GIP and GLP-1 might exert biological effects in various organs, such as the brain, bone, fat and liver, the normal functions of which have been affected by diabetes. We summarize the effects of GIP and GLP-1 in the organs that are likely to be of significance in the treatment of diabetes.

Effects on Adipose Tissues

Obesity and overweight are closely associated with the incidence of diabetes^{138–141} and glycemic control in diabetic patients¹⁴². In addition, obesity has been linked to chronic inflammation that leads to vascular complications^{143,144}. It has been shown that GIP plays physiological roles in nutrient uptake directly into adipose tissues, thereby linking overnutrition to obesity (Figure 5)¹⁴⁵. In contrast, although GLP-1RA is known to reduce bodyweight and body fat¹⁴⁶, GLP-1 seems to have no direct effect on adipocytes. We summarize the effects of GLP-1 and GIP on the adipose tissues and obesity that might have potential clinical significance in the control of bodyweight.

Regarding effects of GIP on adipocytes, an initial clue came in the early 1980s from an experiment showing that GIP, in the presence of insulin, induces fatty acid incorporation into rat epididymal fat pads¹⁴⁷. Later, GIPR was shown to be expressed in adipose tissues¹⁴⁸, and genetic disruption of GIPR further shows the critical role of GIP in fat accumulation¹⁴⁹. Although mice chronically fed on high-fat diets show an increase in

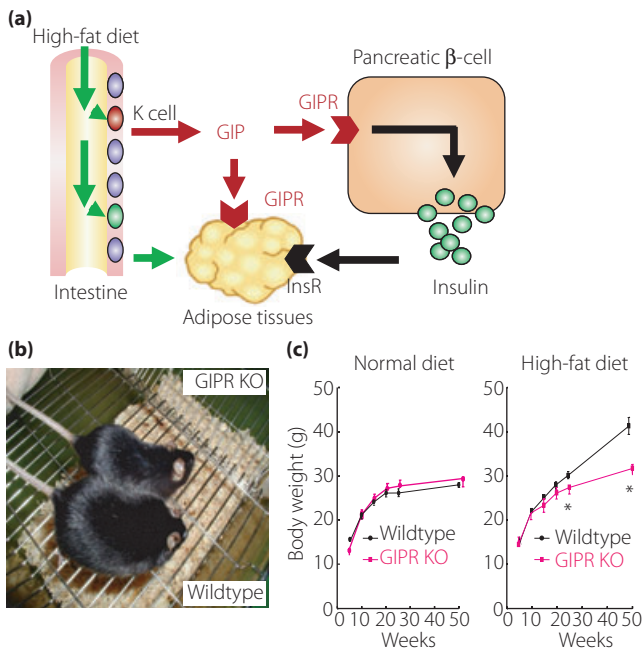


Figure 5 | Effect of glucose-dependent insulinotropic polypeptide (GIP) on fat accumulation. GIP plays physiological roles in nutrient uptake directly into adipose tissues, thereby linking overnutrition to obesity. (a) GIP, in collaboration with insulin whose secretion is also enhanced by GIP, promotes fat accumulation. A recent study showed that GIP receptor (GIPR) in adipose tissues alone is not sufficient for fat accumulation¹⁵⁶. InsR, insulin receptor. (b) Gross appearance of GIPR-deficient mice (GIPR KO) and wild-type mice (Wildtype) fed on a high-fat diet¹⁴⁹. (c) Comparison of bodyweight changes in GIPR KO and Wildtype on either a normal diet or high-fat diet. Note that GIPR KO and Wildtype showed a significant difference in bodyweight changes when they were on a high-fat diet, but not on a normal diet¹⁴⁹. Reproduced from Miyawaki *et al.*¹⁴⁹.

bodyweight as well as visceral and subcutaneous fat mass, such bodyweight gain and fat accumulation was not observed in GIPR-deficient mice fed the same high-fat diet¹⁴⁹. GIPR-deficient mice on high-fat diets showed higher energy expenditure with a reduction of oxygen consumption and respiratory quotient during the light phase, the latter indicating that fat is utilized as the preferred energy substrate¹⁴⁹. In addition, GIPR-deficient mice show increased adiponectin secretion, which promotes fat oxidation in muscle and increases the respiratory quotient^{150,151}. Genetic ablation of GIPR in diabetic *ob/ob* mice prevents obesity by increasing energy expenditure, and improves insulin sensitivity and glucose tolerance without seriously affecting insulin secretion^{145,149}. These observations were reproduced in high-fat fed mice and obese *ob/ob* mice treated with a GIPR antagonist, (Pro3)GIP^{152–154}, and in mice lacking GIP-secreting K cells¹⁵⁵, establishing the critical role of GIP in fat accumulation. Transgenic rescue of GIPR in GIPR-deficient mice suggested that GIP, in collaboration with

insulin, facilitates fat accumulation¹⁵⁶. Although a recent report that GIP-overexpressing mice resulted in reduced diet-induced obesity while they showed enhanced β -cell function and improved glucose tolerance¹⁵⁷ appeared to be inconsistent with these lines of evidence, it was speculated that overexpression of hypothalamic GIP decreased energy intake, resulting in reduced adiposity.

GIP increases the activity of lipoprotein lipase (LPL), an enzyme bound to the cell surface of adipocytes that hydrolyzes lipoprotein-associated triglycerides to produce free fatty acids available for local uptake. It was shown that activation of GIPR in 3T3-L1 cells and rat epididymal fats results in enhanced resistin secretion through a pathway involving p38 mitogen-activated protein kinases (MAPK) and Jun amino-terminal kinase (JNK)¹⁵⁸. GIP activates phosphoinositide 3-kinase and Akt1 through secreted resistin, thereby suppressing adenosine monophosphate-activated protein kinase (AMPK) and increasing LPL activity in adipocytes^{158,159}. It has been also shown that GIP reduces expression and activity of 11 β -hydroxysteroid dehydrogenase type 1, and inhibits the release of free fatty acids in 3T3-L1 cells and healthy humans¹⁶⁰, providing another mechanism underlying GIP-dependent fat accumulation in adipose tissues. Furthermore, it has been shown that GIP activates expression of GIPR through upregulation of peroxisome proliferator-activated receptor (PPAR)- γ ¹⁶¹, suggesting feed-forward action of GIP signaling in adipose tissues. In addition, a recent study has shown that GIP potentiates adipocyte insulin sensitivity of adipocytes through the activation of cAMP/PKA/cAMP response element binding protein signaling module and p110 phosphoinositid-3 kinase, suggesting a feed-forward interaction of GIP with insulin signaling. Furthermore, it has been shown that GIP also enhances glucocorticoid secretion GIPR-dependently, thereby promoting adiposity¹⁶². It is of note that GIP might directly enhance secretion of inflammatory cytokines (e.g., IL-6, TNF- α and MCP-1)¹⁶³, which could worsen vascular complications.

GLP-1 does not show any role in fat accumulation. Although GLP-1R is expressed in adipocytes¹⁶⁴, activation of GLP-1R does not increase LPL activity in adipocytes^{158,159}. Recently, it has been shown that GLP-1 directly activates adipogenesis through activation of PKC, ERK and Akt1, which leads to altered proliferation, apoptosis and differentiation¹⁶⁵. However, the physiological relevance of this finding is unknown and needs to be determined in future.

Effects on Food Intake and Gastric Emptying

It has been shown that GLP-1RA reduces bodyweight likely by inhibiting food intake and slowing gastric emptying^{146,166,167}. We summarize the effects of GLP-1 and GIP on food intake and gastric emptying that might have effects on overweight.

GLP-1 has been shown to be expressed in the arcuate nucleus and other hypothalamic regions involved in the regulation of food intake¹⁶⁸. Subcutaneous and intracerebroventricular GLP-1 injections were shown to significantly inhibit food intake

and bodyweight in non-diabetic rats, and this inhibitory effect on the food intake was blocked by GLP-1R antagonist exendin (9-39)¹⁶⁹. Intraperitoneal injection of GLP-1RA liraglutide and exendin-4 also reduced food intake in non-diabetic rats¹⁷⁰. In addition, subcutaneous injections of liraglutide significantly reduced the food intake and bodyweight in diabetic *ob/ob* and *db/db* mice¹⁷¹, as well as in obese candy-fed rats¹⁷², obese mini-pigs¹⁷³ and obese rats carrying hypothalamic lesions¹⁷⁴. It was also shown that GLP-1 or GLP-1RA liraglutide infusion reduced food intake and reduced gastric emptying in healthy volunteers¹⁷⁵, patients with type 1 diabetes¹⁷⁶ and patients with type 2 diabetes^{177,178}.

The doses of exendin-4 required for significant reduction of food intake were higher in subdiaphragmatically vagotomized rats than in non-vagotomized rats¹⁷⁹. This result suggests that the reduction of the food intake noted after administration of exendin-4 might be mediated by activation of the GLP-1 expressed on the vagal afferents, as well as by direct activation of GLP-1R in the central nervous system. In contrast, direct injection of exendin(9-39) into the nucleus tractus solitarius in rats significantly increased the food intake¹⁸⁰. Blockage of hind-brain GLP-1R by exendin(9-39) inhibited the suppression of the food intake induced by gastric distention, but did not affect the suppression of the food intake induced by intraduodenal nutrient infusion. These results suggest that nucleus tractus solitarius activation by GLP-1 might also contribute to the control of food intake through the satiating effects of gastric distension. A recent study showed that GLP-1R activation in the nucleus tractus solitarius neurons increased PKA activity concomitant with an increase in phosphorylation of p44/42-MAPK and a decrease in phosphorylation of AMPK¹⁸¹. The combined increase in PKA and p44/42-MAPK activity, together with decreased AMPK activity, is hypothesized to increase cAMP-response element binding protein-mediated nuclear transcription and protein synthesis, resulting in reduced food intake¹⁸¹. Furthermore, it has been reported recently that GLP-1 neurons in the nucleus tractus solitarius also projects directly to the ventral tegmental area and nucleus accumbens, the site in which GLP-1 is thought to regulate food intake¹⁸². Regarding gastric emptying, intracisternal GLP-1 injection induced delayed gastric emptying, an effect that was partially antagonized by celiac ganglion-ectomy, but not by atropine or NOS inhibitor, L-NG-nitroarginine methyl ester (L-NAME)¹⁸³. Corticotropin-releasing hormone antagonist, astressin, also partially antagonized the GLP-1-induced delay of gastric emptying¹⁸³. These observations show that central corticotropin releasing hormone and peripheral sympathetic pathways might mediate the delay of gastric emptying induced by centrally administered GLP-1. It is clinically of interest that chronic infusion of GLP-1 or the relatively long-acting GLP-1RA, liraglutide, but not the short acting GLP-1RA, exenatide, loses its effects on gastric emptying rapidly as a result of tachyphylaxis in patients with diabetes and in rats^{184,185}. Although GLP-1 reduces the food intake and delays gastric emptying in experimental animals and humans,

GIP does not have such effects in humans, but rather facilitates gastric emptying^{175,186}. It is noteworthy that although DPP-4i sitagliptin has no effects on gastric emptying, the GIP response negatively correlates with gastric emptying and the GLP-1 response positively correlates with gastric emptying¹⁰⁸. A recent report showed that exogenous GIP inhibits intestinal motility through a somatostatin-mediated pathway rather than through a GLP-1-mediated pathway, thereby inhibiting glucose absorption¹⁸⁷.

Effect on the Liver

Diabetes is closely associated with non-alcoholic fatty liver disease (NAFLD), the prevalence of which is 10–30% depending on geographical region. NAFLD is caused by impaired hepatic lipid homeostasis that is usually maintained through a balance between the influx or production of fatty acids and their use for oxidation or secretion as very low-density lipoprotein triglycerides^{188–190}. Approximately 10–25% of individuals with NAFLD progress to non-alcoholic steatohepatitis (NASH), a silent disease with few or no symptoms, and eventually develops liver cirrhosis and hepatocellular carcinoma. Several clinical trials showed that agents, such as pioglitazone, vitamin E, ursodeoxycholic acid and L-carnitine, exert beneficial effects against NAFLD. However, reducing bodyweight by appropriate diet and exercise therapies is still considered superior to any pharmacological therapies today. It has been shown that GLP-1R activation results in amelioration of hepatic lipid accumulation^{102,103,191–193}. In contrast, GIP has been associated with fat accumulation in the liver of high-fat fed animals, but GIPR expression in the liver is controversial^{149,152,154,194}. We summarize the effects of GLP-1 and GIP on the liver that might have potential clinical significance in the treatment of NASH and NAFLD.

Although GLP-1R expression in the mouse liver or hepatocytes is controversial, exendin-4 reversed high-fat induced hepatic lipid accumulation and inflammation in hepatocytes and C57BL/6J mice¹⁹¹. Exendin-4 also reversed hepatic steatosis in *ob/ob* mice¹⁰². In the same study, GLP-1-treated mouse hepatocytes resulted in a significant increase in cAMP production along with reduced expression of stearoyl-CoA desaturase (SCD)-1 and genes associated with fatty acid synthesis, and increased expression of genes involved in fatty acid oxidation¹⁰². It was also shown that incubation of rat hepatocytes with exendin-4 increased expression of PPAR- α along with their downstream genes, acyl-coenzyme A oxidase and carnitine palmitoyltransferase 1A, thereby reducing intracellular fatty acids¹⁹². Similarly, it was shown that another GLP-1RA liraglutide significantly ameliorates lipid accumulation by increasing expression of genes related to fatty acid transport and beta-oxidation in mice on high-fat and high-fructose diets¹⁰³. In addition to improvement of lipid metabolisms, exendin-4 has been shown to improve hepatic insulin sensitivity. Administration of exendin-4 increased expression of Sirt1 along with AMPK in livers of high-fat fed C57BL/6J mice, as well as in cultured

hepatic cell lines, HepG2 and Huh7 cells, suggesting involvement of GLP-1 signaling not only in lipid metabolism, but also in glucose metabolism in the liver. Another study showed that incubation of rat hepatocytes with exendin-4 increased expression of PPAR- γ , which exerted its insulin-sensitizing action by reducing JNK phosphorylation, and increased phosphorylation of Akt1 and AMPK¹⁹². Similarly, it was shown that exendin-4 increased the phosphorylation of Akt1 and PKC- ζ in HepG2 and Huh7 cells¹⁹⁵. A recent study also showed that exendin-4 activates glucokinase and increases hepatic glycogen contents in streptozotocin (STZ)-treated C57BL/6J mice independently of insulin¹⁹⁶. Collectively, GLP-1RA is supposed to ameliorate insulin sensitivity and regulate glucose metabolism in the liver. Another effect of GLP-1RA in the liver includes protection against hepatocellular injuries. It was shown that exendin-4 exerts a protective role in the steatotic liver by inhibiting cell death through upregulation of genes associated with autophagy, thereby reducing endoplasmic reticulum (ER) stress-related apoptosis in human hepatocytes treated with fatty acids, as well as in mice fed a high-fat diet¹⁹³. Furthermore, exendin-4 administration in STZ-treated diabetic mice showed that exendin-4 suppresses hepatocyte injury by decreasing proliferation of hepatocytes¹⁹⁷, which might have implications in treatment of NAFLD. Indeed, it was shown that exenatide treatment ameliorates hepatic biomarkers in patients with type 2 diabetes treated for at least 3 years¹⁹⁸. Whether the liver or hepatocytes in humans express GLP-1R needs to be addressed cautiously, as it is possible that effects of GLP-1 in the liver are partly mediated through the nervous system, as evidenced in mice.

In contrast to GLP-1, GIP was associated with fat accumulation in the livers of high-fat fed animals, but GIPR expression in the liver is still controversial. It was shown that GIP antagonist (Pro3)GIP not only reduces bodyweight, but also ameliorates fat accumulation in the livers of high-fat fed mice^{152,154} and *ob/ob* mice¹⁵³. These findings were further established by recent a investigation in high-fat fed mice showing that active immunization against (Pro3)GIP resulted in GIP antibody production and significant reduction of liver triglyceride along with reduction in blood glucose levels¹⁹⁴. These lines of evidence suggest that GIP antagonism might be beneficial as treatment for NASH and NAFLD. However, this was challenged by a recent report that GIP-overexpressing mice resulted in reduced diet-induced obesity and amelioration of liver steatosis, whereas they showed enhanced β -cell function and improved glucose tolerance¹⁵⁷. Although this finding by McIntosh *et al.*¹⁵⁷ was unexpected from the results on GIPR-deficient mice that also showed reduced diet-induced obesity, they speculated that overexpression of hypothalamic GIP decreased energy intake, resulting in reduced adiposity¹⁵⁷. Further studies are required to develop GIP-related therapies for obesity and NAFLD.

Effects of DPP-4 inhibition have been investigated in mice and humans. DPP-4 inhibition in diet-induced diabetic glucoki-

nase^{+/-} mice suppressed hepatic inflammation and reduced expression of sterol-regulatory binding protein (SREBP)-1c, SCD-1 and fatty acid synthase (FAS), and upregulation of PPAR- α , thereby ameliorating hepatic steatosis¹⁹⁹. Similarly, DPP-4 inhibition improves insulin sensitivity and hepatic steatosis in diet-induced obese C57BL/6 mice with reduced hepatic expression of SREBP-1c, SCD-1 and FAS²⁰⁰. Furthermore, it was shown that DPP-4i sitagliptin ameliorates hepatic biomarkers in type 2 diabetic patients with NAFLD treated for 4 months²⁰¹. In addition, sitagliptin was shown to ameliorate liver enzymes and hepatocyte ballooning in type 2 diabetic patients with NASH²⁰². Taken together, these results suggest clinical implications of DPP-4i in treatment for NASH and NAFLD.

Effect on the Brain

Dementia has been postulated as one of the diabetic comorbidities²⁰³. GIPR and GLP-1R have been found to be expressed in the hippocampus, which is the brain region most closely involved in memory formation^{169,204}. GIPR-deficient mice are impaired in learning, synaptic plasticity and hippocampal neurogenesis⁸⁴, whereas GIP and GIPR agonist, N-AcGIP, has been shown to strongly enhance hippocampal long-term potentiation (LTP)⁸⁵. GLP-1R-deficient mice were also found to be impaired in a memory-related behavioral task, and hippocampal LTP was severely impaired²⁰⁵, whereas exendin-4 administration improved cognitive function in adult mice²⁰⁶, and enhanced neurogenesis in the hippocampus of diabetic and non-diabetic mice²⁰⁷.

Injection of β -amyloid (A β), which is well-known to accumulate in the brain of Alzheimer's disease (AD) patients, impaired LTP; N-AcGIP fully reversed the impairment of LTP induced by the injection of A β ⁸⁵. GLP-1 and GLP-1RA also exert a protective role in a transgenic mouse model of AD^{208,209}. GLP-1 protected against the cellular apoptosis induced by A β ²¹⁰. GLP-1 has the ability to reduce the levels of A β in the brain *in vivo*, and also reduces the levels of the amyloid precursor protein in cultured neuronal cells²¹¹. Furthermore, GLP-1 and exendin-4 protected cultured hippocampal neurons against apoptosis induced by A β and oxidative insults²¹¹. Furthermore, GLP-1R activation allows physiological tyrosine phosphorylation of IRS (insulin receptor substrate)-1 and stimulation of downstream insulin signaling by inhibition of A β oligomer-activated JNK²¹². A recent study has shown that exendin-4 restored dopamine and noradrenaline contents, and reversed neurological dysfunction in a mouse model of Parkinson's disease²¹³. The neuroprotective effects of exendin-4 on hyperglycemia- and LPS-induced cognitive dysfunction also were shown²¹⁴. Furthermore, GLP-1RA (Val8)GLP-1 prevents tau hyperphosphorylation, impairment of spatial learning and ultrastructural cellular damage in STZ-treated rat brains²¹⁵.

These findings suggest that GIP and GLP-1 might also play important roles in the control of synaptic plasticity and memory formation, and that GIP and GLP-1 analogs might well

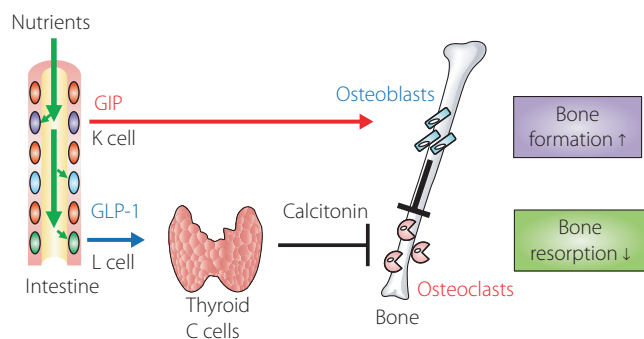


Figure 6 | Regulation of bone metabolism by glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). The effects of GIP and GLP-1 on bone metabolism. GIP binds to GIP receptors expressed on osteoblasts, thereby activating new bone formation. GIP also acts on osteoclasts, presumably through osteoblasts, to suppress bone resorption. In contrast, GLP-1 stimulates calcitonin secretion from the thyroid gland, which then suppresses bone resorption by osteoclasts. Notably, it has been reported that GLP-1 receptor expression in humans is much lower than in rodents²⁴⁰. Reproduced from Yabe and Seino²¹⁷.

inhibit impairment of memory formation in patients with AD, as well as other cognitive deficits in neurodegenerative disorders.

Effect on the Bone

Bone fracture, which is directly related to decreased mobility and compromised quality of life (QOL), is associated with diabetes²¹⁶. It has been reported that GIP promotes bone formation, whereas GLP-1 inhibits bone resorption (Figure 6)^{217,218}. Thus, both GIP and GLP-1 could play important roles in the reduction of the bone fracture risk in diabetic patients.

GIPR has been shown to be present in osteoclasts. GIP inhibits the osteoclast resorptive activity in organ culture systems and the resorptive activity of mature osteoclasts²¹⁹. The bone formation parameters in GIPR-deficient mice were significantly lower than those in wild-type mice, and the number of osteoclasts was significantly increased in GIPR-deficient mice, indicative of high-turnover osteoporosis²²⁰. In addition, GIP suppressed the apoptosis of osteoblasts *in vitro*, suggesting that GIP stimulates bone formation through inhibition of osteoblast apoptosis²²⁰. Furthermore, GIP transgenic mice showed a significant increase in the markers of bone formation and a decrease in the markers of bone resorption, and also a significant increase of the bone mass^{221,222}. From these findings in GIPR-deficient mice and GIP-transgenic mice, it is suggested that GIP inhibits bone resorption and stimulates bone formation, and that excess signaling through the GIPR results in the gain of bone mass. In addition, the plasma calcium concentration after meal ingestion in GIPR-deficient mice was increased, and GIP might play an important role in the stimulation of calcium deposition in the bone²²³.

GLP-1R-deficient mice showed cortical osteopenia, bone fragility and an increase in the markers of bone resorption and osteoclast numbers²²⁴. GLP-1R-deficient mice also showed reduced calcitonin gene expression in the thyroid, whereas exendin-4 increased calcitonin expression. GLP-1 did not show any direct effect on the osteoblasts or osteoclasts, although it appears to have an essential role in endogenous GLP-1R signaling in bone resorption through activation of the calcitonin-dependent pathway, which in turn, plays an important role in bone formation²²⁴. In the analysis of bone structure in STZ-induced diabetic rats and fructose-induced insulin resistance rats, GLP-1 significantly reduced the trabecular separation²²⁵, whereas exendin-4 induced a significant decrease in trabecular separation, and increase in trabecular thickness and trabecular spacing. It also has been also shown that GLP-1 and exendin-4 reversed hyperlipidic-related osteopenia²²⁶. Exendin-4 normalized the LDL-receptor-related protein 5 (an activator of the wntless type [Wnt] pathway)/Dickkopf-related protein 1 (a blocker of LDL-receptor-related protein 5) ratio in diabetic rats and insulin resistant rats²²⁷. This finding suggests that exendin-4 might induce the bone formation in diabetic and insulin resistant rats through the Wnt signaling pathway²²⁷. Consistent with the effects of GIP and GLP-1 on bone metabolism, DPP-4i sitagliptin significantly improved vertebral volumetric bone mineral density and trabecular architecture in female mice²²⁸.

Although these lines of evidence suggest an association of GIP and GLP-1 with bone metabolism in humans, the effects of GIP and GLP-1 on human bone turnover are largely unknown. A meta-analysis of 28 clinical trials of treatment with DPP-4i (i.e., vildagliptin, sitagliptin, saxagliptin, alogliptin, linagliptin and dutogliptin for at least 24 weeks) in patients with type 2 diabetes showed reduced the risk of bone fractures in patients treated with DPP-4i²²⁹. However, 44-week exenatide treatment did not affect the bone mineral density or serum markers of bone homeostasis (i.e., serum alkaline phosphatase, calcium and phosphate), as compared with insulin glargine, in patients with type 2 diabetes²³⁰. As mentioned, GLP-1 action on the bone is presumably mediated through calcitonin. A series of clinical trials on liraglutide showed few changes in serum calcitonin levels in patients with type 2 diabetes, suggesting that GLP-1 might not play a role in human bone metabolism. Regarding GIP, it has been previously reported that postprandial reduction of bone resorption was not mediated by GIP, but by GLP-2, another intestinal hormone secreted simultaneously with GLP-1²³¹. However, they investigated the effects of subcutaneous single injections of native GIP that should be rapidly inactivated by DPP-4 before it reached the bones. Thus, further investigations are definitely required to understand GIP and GLP-1 actions on bone metabolism in humans.

Effects on Thyroid and Tumorigenesis

Studies in experimental animals, including toxicology studies, did not show that incretins had an effect on tumorigenesis. However, it has been recently reported that GLP-1RA liraglutide and exe-

natide were associated with benign and malignant thyroid tumors in rats and mice²³². GLP-1R was found to be expressed in normal rat thyroid, as well as medullary thyroid carcinoma cells²³³. GLP-1 and GLP-1RA stimulate expression and secretion of calcitonin, a clinical biomarker for C cell diseases, such as medullary thyroid carcinoma²³⁴, in a dose-dependent manner along with production of cAMP in rodent C cell lines^{232,233,235} and rodents^{232,236}. However, it was reported that GLP-1R expression was low in a human C cell line, and that liraglutide enhances little calcitonin secretion or C cell proliferation in primates²³². Furthermore, clinical trials of GLP-1RA, such as liraglutide, did not report elevation of calcitonin^{237,238}. Thus, although the preclinical studies in rodents show a link between GLP-1 and thyroid tumors, the relevance of these findings for humans is still unclear. In addition to the marked differences in the way that rodent and human C cells respond to GLP-1, spontaneous C cell tumors are frequently seen in rats, whereas medullary thyroid cancer in humans is rare. Nevertheless, a recent study indicates expression of GLP-1R in neoplastic and hyperplastic lesions of thyroid C cells²³⁹. Thus, long-term observational studies are required to monitor the effects of sustained GLP-1R signaling over the long term on the human thyroid.

CONCLUSION

Biological processes regulated by the incretin hormones, GIP and GLP-1, are much broader than previously expected. Their insulinotropic actions are applied in the development of incretin-based therapies, DPP-4i and GLP-1RA that exert glucose lowering effects, thereby suppressing diabetes-related complications in patients with type 2 diabetes. In addition, it is conceivable that extrapancreatic function of GIP and GLP-1 might be exploited to prevent onset and progression of diabetes-related complications independently of glycemic control. A series of experimental results obtained so far suggest that diabetes-related microvascular complications (e.g., retinopathy, nephropathy and neuropathy) and macrovascular complications (e.g., CAD, CVD and PAD) are directly affected by incretin-based therapies. Furthermore, diabetes-related comorbidities, such as cognitive dysfunction, obesity, fatty liver and bone fracture, might be also ameliorated by incretin-based therapies. Clinical trials with adequately powered, prospective, controlled relevant end-points will clarify, in future, the effects of incretin-based drugs on diabetes-related complications.

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