REVIEW ARTICLE

GIP and GLP-1, the two incretin hormones: Similarities and differences

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ABSTRACT

Gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are the two primary incretin hormones secreted from the intestine on ingestion of glucose or nutrients to stimulate insulin secretion from pancreatic β cells. 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. In addition to their insulinotropic effects, GIP and GLP-1 play critical roles in various biological processes in different tissues and organs that express GIPR and GLP-1R, including the pancreas, fat, bone and the brain. Within the pancreas, GIP and GLP-1 together promote β cell proliferation and inhibit apoptosis, thereby expanding pancreatic β cell mass, while GIP enhances postprandial glucagon response and GLP-1 suppresses it. In adipose tissues, GIP but not GLP-1 facilitates fat deposition. In bone, GIP promotes bone formation while GLP-1 inhibits bone absorption. In the brain, both GIP and GLP-1 are thought to be involved in memory formation as well as the control of appetite. In addition to these differences, secretion of GIP and GLP-1 and their insulinotropic effects on β cells have been shown to differ in patients with type 2 diabetes compared to healthy subjects. We summarize here the similarities and differences of these two incretin hormones in secretion and metabolism, their insulinotropic action on pancreatic β cells, and their non-insulinotropic effects, and discuss their potential in treatment of type 2 diabetes. (J Diabetes Invest, doi: 10.1111/j.2040-1124.2010.00022.x, 2010)

KEY WORDS: Incretin, GIP, GLP-1

INTRODUCTION

Over 100 years have passed since the discovery of the incretin concept, which later opened up the possibility of a novel therapy in the treatment of diabetes. Inspired by Bayliss and Starling's discovery of secretin in 1902[1], Moore *et al.* hypothesized that gut extracts contain a hormone that regulates the endocrine pancreas, and showed that administration of gut extracts reduces the amount of urine sugars in patients with diabetes, presumably through stimulation of the endocrine pancreas[2]. In 1929, La Barre purified the glucose-lowering element from gut extracts, and named it incretin (INtestine seCRETtion Insulin)[3]. However, incretin was forgotten for three decades until radioimmunoassay to measure insulin became available in the 1960s. Oral glucose load was shown to produce a much greater insulin response than i.v. injection of glucose[4,5], which now can be attributed to incretins released from the gut after ingestion of glucose or nutrients to stimulate insulin secretion from pancreatic β cells.

Merely two such gut hormones, gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), have been shown to act as incretins (Figure 1). GIP is a 42-amino-acid hormone secreted from K cells of the upper small intestine[6,7]. It was originally isolated from porcine intestine on the basis of its ability to inhibit gastric acid secretion[8]. Later, it was found that GIP administration stimulates insulin secretion in healthy volunteers[9], and that GIP acts directly on pancreatic islets to stimulate insulin secretion[10,11]. We have also shown that endogenous GIP stimulates insulin secretion glucose-dependently in gastrectomized patients[12]. These lines of evidence showed GIP to be the first incretin, which was then renamed glucose-dependent insulinotropic polypeptide. Because immunological depletion of GIP did not abolish all insulin-stimulating activity in gut extracts[13], the existence of a second incretin was inferred. Meanwhile, a series of investigations on enteroglucagons showed that GLP-1, a 31-amino-acid hormone produced from proglucagon and secreted from L cells of the lower intestine and colon[14], directly acts on islets and stimulates insulin secretion in isolated islets[15] as well as in healthy volunteers[16]. GLP-1 was thus found to be the second incretin.

Both GIP and GLP-1 exert their effects by binding to their specific receptors, the GIP receptor (GIPR)[17–21] and the GLP-1 receptor (GLP-1R)[22–24], which belong to the G-protein coupled receptor family, activating adenylate cyclase and increasing levels of intracellular cyclic adenosine monophosphate (cAMP) in pancreatic β cells, thereby stimulating insulin section 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 both GIP and GLP-1 act

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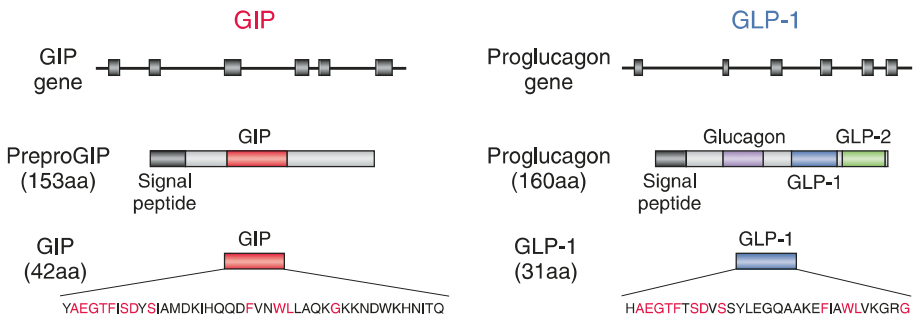

Figure 1 | The glucose-dependent insulinotropic polypepide (GIP) gene is localized on human chromosome 17q21.3–q22 and comprises 6 exons. Proteolytic processing of preproGIP generates GIP that is secreted from K cells. The proglucagon gene is localized on human chromosome 2q36–q37 and comprises 6 exons. In the intestine, proteolytic processing of proglucagon generates glucagon-like peptide (GLP)-1 and GLP-2, whereas glucagon is produced in the pancreas.

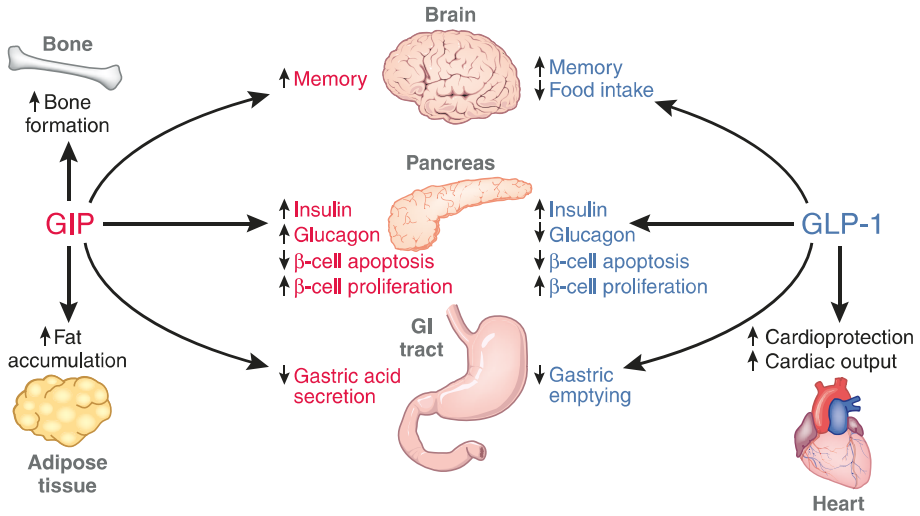

Figure 2 | Pancreatic and exopancreatic function of glucose-dependent insulinotropic polypepide (GIP) and glucagon-like peptide (GLP)-1. GIP acts directly on the endocrine pancreas, bone, fat, gastrointestinal (GI) tract and brain. GLP-1 acts directly on the endocrine pancreas, gastrointestinal tract, heart and brain.

as incretins[25–29]. Furthermore, deficiency of dipeptidyl peptidase-4 (DDP-4), which cleaves the two NH_2-terminal amino acids of GIP and GLP-1 in plasma and inactivates their insulinotropic activities[30,31], enhances insulin secretion in response to oral glucose challenge consistently with their function as incretins[32]. GIP and GLP-1 thus share common properties as incretins, but they also possess different biological characteristics (Figure 2). Here, we summarize similarities and differences in the processes of the secretion and metabolism of GIP and GLP-1, their insulinotropic actions on pancreatic β cells, and their non-insulinotropic effects.

SECRETION AND METABOLISM OF GIP AND GLP-1

Because GIP and GLP-1 rapidly undergo proteolytic degradation catalyzed by DPP-4[30,31], not only intact but also total (i.e. intact plus DPP-4-metabolized) forms of GIP and GLP-1 must be measured to study their secretion and processing *in vivo* (Figure 3). However, immunoassays for GIP and GLP-1 levels, especially those used to measure their intact forms in plasma, require specific antibodies and are not widely available[33]. Furthermore, because carboxyl-terminal arginine of GLP-1 is susceptible to amidation, GLP-1 occurs in both non-amidated GLP-1(7–37) and amidated GLP-1(7–36)amide, both of which show similar insulinotropic effects and metabolism in humans[34]. Although most of the GLP-1 secreted from the gut is amidated in humans[35], careful considerations are required when measuring the levels of GLP-1 because some antibodies only recognize amidated GLP-1.

GIP secretion from K cells is enhanced in response to ingestion of meals or glucose[36]. A series of studies using the antibody R65, which recognizes both intact GIP(1–42) and DPP-4-processed GIP(3–42), shows that plasma levels of total GIP at fasting are 5–20 pM in healthy Caucasians[36], indicating basal secretion in healthy Caucasians. These levels of total GIP reach 50–100 pM within 30 min in response to ingestion of 75-gram glucose in healthy Caucasians, whereas those of total GIP reach 100–150 pM within 60 min in response to ingestion of mixed

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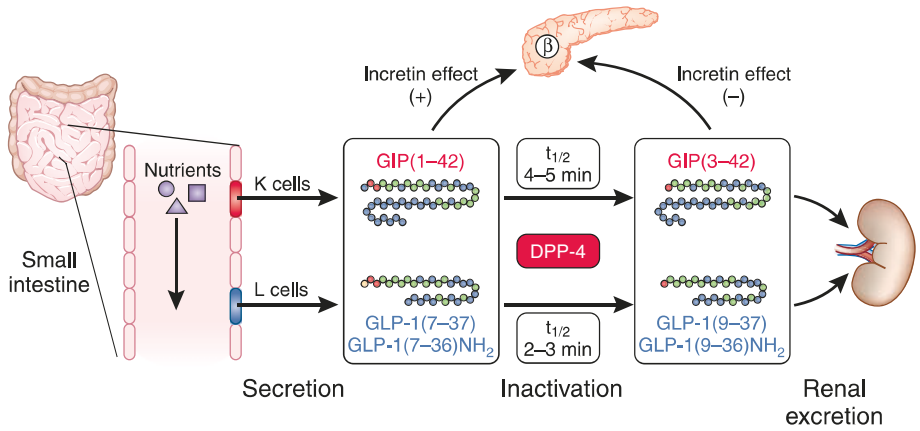

Figure 3 | Secretion and metabolism of glucose-dependent insulinotropic polypepide (GIP) and glucagon-like peptide (GLP)-1. GIP is secreted from K cells of the upper intestine; GLP-1 is secreted from L cells of the lower intestine. Released GIP and GLP-1 rapidly undergoes proteolytic processing by dipeptidyl peptidase-4 (DPP-4), and is thereby inactivated and excreted from the kidney. The intact incretins, GIP(1–42), GLP-1(7–37), and GLP-1(7–36)amide, have insulinotropic effects on pancreatic β cells, whereas the DPP-4-processed incretins, GIP(3–42), GLP-1(9–37), and GLP-1(9–36)amide, have lost their insulinotropic effects.

meals[36,37]. Although there is no direct comparison of glucose-enhanced GIP secretion with those enhanced by proteins or fats, ingestion of proteins produces more rapid and robust GIP secretion than that of fats[38]. Similarly, levels of intact GIP, determined by an immunoassay using antiserum 98171, which detects GIP(1–42) but not GIP(3–42)[39], increase more rapidly and robustly in response to ingestion of proteins when compared with isocaloric fat ingestion[38]. These results suggest that the GIP response is dependent not only on meal size but also on meal composition. Recent investigations of GIP secretion in healthy Japanese subjects using the same immunoassays showed that although peak values of total GIP levels in response to ingestion of glucose or mixed meals are higher than those of Caucasians, the peak values of intact GIP levels are similar[37,40–42], suggesting enhanced processing of GIP by DPP-4 in Japanese subjects (Figure 4). This possible racial difference in the GIP response and DPP-4 activities needs to be studied more intensively in the future.

GLP-1 secretion from L cells, like that of GIP from K cells, is enhanced in response to ingestion of meals or glucose[36]. Studies using antiserum 89390 specific for GLP-1(7–36)amide as well as DPP-4-processed GLP-1(9–36)amide show that plasma levels of total GLP-1 at fasting are 10–20 pM, indicating basal secretion in healthy Caucasians[36]. Levels of total GLP-1 reach 30–60 pM within 30 min in response to ingestion of 75-gram glucose or mixed meals in healthy Caucasians[36,37]. Despite the lack of direct comparison of glucose-enhanced GLP-1 secretion with those enhanced by proteins or fats, ingestion of proteins or isocaloric fats produces a similar GLP-1 secretion[38]. The levels of intact GLP-1 are controversial; recent studies have shown the importance of an ethanol or solid phase extraction before immunoassays for intact GLP-1 that removes interference with substances of unknown identity and thereby reduces the large variability among individual human subjects[33]. Evaluation of GLP-1 secretion in healthy Japanese subjects using the same immunoassay detecting total GLP-1 with antiserum 89390 showed that the meal-induced enhancement of GLP-1 secretion is negligible, whereas GLP-1 secretion in response to oral glucose is similar to that in healthy Caucasians[36,37,40–42]. The levels of intact GLP-1 in ethanol extracted plasmas, determined by an immunoassay with the monoclonal antibodies GLP1F5 and Mab26.1, were considerably low in Japanese subjects, and showed no enhancement in response to glucose or mixed meal ingestion[42]. Although further studies to directly compare the GLP-1 response of Asian and Caucasian subjects are required, the blunted meal-induced enhancement of the GLP-1 response and the considerably low levels of intact GLP-1 could well account for the reduced insulin secretory capacity of Asians, including Japanese.

Secreted incretins undergo rapid degradation catalyzed by DPP-4, which diminishes the insulinotropic effects of GIP and GLP-1[43]. The apparent half-lives for intact GIP and GLP-1 have been determined as approximately 5 and 2 min, respectively[40,43,44]. Studies of GLP-1 secreted from perfused pig ileum show that approximately 75% of GLP-1 leaving the gut is already metabolized by DPP-4[45]. Further degradation of GLP-1 also occurs in the liver, which finally results in only 10–15% of newly secreted GLP-1 in systemic circulation[46]. Furthermore, study of intact GLP-1 levels in Japanese subjects shows that less than 5% of intact GLP-1 reaches systemic circulation (Figure 4), suggesting that GLP-1 acts on putative GLP-1R in the portal vein and then stimulates insulin secretion through activation of the vagus nerve. Contribution of portal GLP-1R activation in insulinotropic function of GLP-1 could be evaluated in the future using mice lacking specifically GLP-1R in the portal vein.

In both Caucasians and Japanese with type 2 diabetes (T2DM), the GIP response is enhanced compared with that in healthy volunteers[47,48], whereas the GLP-1 response in Caucasians with T2DM is reduced compared with that in healthy

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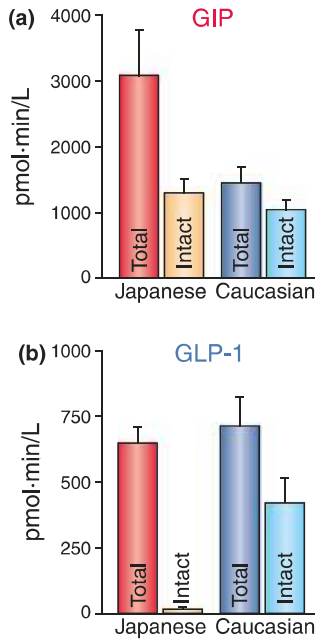

Figure 4 | Racial differences in secretion and metabolism of (a) glucose-dependent insulinotropic polypepide (GIP) and (b) glucagon-like peptide (GLP)-1. GIP secretion in healthy Japanese subjects after ingestion of glucose or mixed meals were higher than those of healthy Caucasian subjects, whereas levels of intact GIP were similar[37,40–42]. This suggests processing of GIP by dipeptidyl peptidase-4 (DPP-4) in Japanese subjects might be enhanced. Considerably low levels of intact GLP-1 after ingestion of glucose or mixed meals are also consistent with enhanced DPP-4 activities in Japanese subjects. To compare early phase incretin secretion and their processing by DPP-4 between non-obese (BMI <25) healthy Japanese and Caucasian subjects, area under the curves during 0–30 min after ingestion of mixed meals containing similar calories are plotted for total and intact forms of (a) GIP and (b) GLP-1 from Yabe *et al.*[42] Note that total and intact GIP and total GLP-1 levels were measured in the same immunoassays, whereas assays for intact GLP-1 were similar except for the presence or absence of the ethanol extraction.

controls[37]. The physiological cause for the enhanced GIP response in patients with T2DM is not yet clear. Furthermore, the incretin response in T2DM patients is still controversial, because Caucasian and Japanese patients with a relatively short duration of diabetes show GIP and GLP-1 response similar to those of healthy controls[36,42]. Although it is not discussed in the present review, recent advances in understanding the molecular mechanisms underlying GIP and GLP-1 secretion from K and L cells[49] should clarify the differing results on GIP and GLP-1 responses in T2DM patients in the various studies.

INSULINOTROPIC ACTIONS OF GIP AND GLP-1

Enhancement of glucose-dependent insulin secretion by incretins in healthy volunteers was directly shown by i.v. infusion of GIP[9] and GLP-1[16]; their insulinotropic effects are similar[50] and additive[51] in healthy humans. Critically, the effects of infused GIP at pharmacological levels are impaired in Caucasians with T2DM, although those of GLP-1 are substantially preserved[52,53]. The insulinotropic effects of endogenous GIP are also diminished in Japanese T2DM patients[48]. Furthermore, the impaired insulinotropic action of GIP, but not that of GLP-1, are strikingly similar in perfused islets of diabetic Goto-Kakizaki rats[54]. However, the molecular mechanisms underlying the impaired insulinotropic action of GIP in T2DM patients are still largely unknown. Various studies in model animals suggest downregulation of GIPR mRNA[55–58], accelerated degradation of GIPR[59], and alternative splicing of GIPR mRNA[60], all of which might contribute to the reduced GIP sensitivity of pancreatic β cells. Polymorphisms in the human GIPR gene have been shown to decrease GIP sensitivity in β cells, but no correlation has been observed between these polymorphisms and T2DM[61]. However, genetic variation in GIPR has been recently reported to be associated with the reduction in early phase insulin reaction and elevation in 2-h glucose levels after ingestion of 75-gram glucose, suggesting that defective GIPR signaling might play a critical role in the early pathophysiology of impaired glucose tolerance and T2DM[62]. It remains to be determined why GIP but not GLP-1 signaling is selectively impaired in hyperglycemic conditions in humans.

Both GIP and GLP-1 exert their insulinotropic effects by binding to GIP and GLP-1 receptors expressed on pancreatic β cells. Incretin-bound receptors increase intracellular cAMP levels[63,64], thereby activating protein kinase A (PKA)[65] and exchange protein activated by cAMP2 (EPAC2)/cAMP-guanine nucleotide exchange factor (GEF) II[66]. PKA and EPAC2 are involved in a wide variety of intracellular events including altered ion channel activity, elevated cytosolic calcium levels and enhanced exocytosis of insulin-containing granules, all of which contribute to stimulation of insulin section in a glucose-dependent manner (Figure 5). Many studies have been carried out using inhibitors and activators that affect signal transduction or GLP-1 receptor agonist exentin-4, assuming that the molecular mechanisms downstream of both GIPR and GLP-1R are similar. Activation of PKA results in phosphorylation of the SUR1 subunit, thereby closing the K_{ATP} channels and facilitating membrane depolarization[67]. PKA, together with phosphoinositide 3-kinase (PI-3K), also leads to inhibition of the delayed rectifying K^+ (Kv) channel, which results in prolongation of action potentials[68]. Depolarization opens the voltage-gated Ca^{2+} channels (VDCC), allowing an increase of intracellular Ca^{2+} concentrations, which then mobilizes Ca^{2+} from intracellular stores through PKA- and EPAC2-dependent mechanisms[69–73]. The increased Ca^{2+} concentrations eventually trigger fusion of insulin-containing granules with the plasma membrane and increase insulin secretion from the β cells. Mobilization of Ca^{2+} from intracellular stores stimulates adenosine triphosphate (ATP) synthesis in mitochondria, which further enhances membrane depolarization by K_{ATP} channel closure[74]. Furthermore, activation of EPAC2 has recently been shown to increase the density of insulin-containing granules near the plasma membrane, facilitating insulin secretion from the β cells[75]. Taken together, these lines of evidence show the critical functions of PKA and EPAC2 pathways in the insulinotropic actions of GIP and GLP-1.

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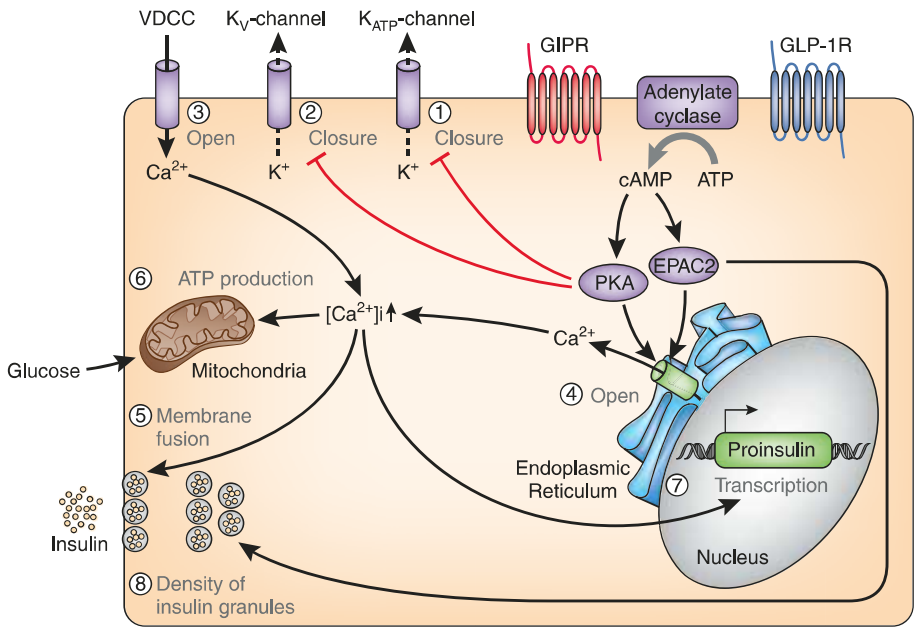

Figure 5 | Molecular mechanisms underlying the insulinotropic effects of glucose-dependent insulinotropic polypepide (GIP) and glucagon-like peptide (GLP)-1. Binding of GIP and GLP-1 to their specific receptors, the GIP receptor (GIPR) and the GLP-1 receptor (GLP-1R) leads to activation of adenylate cyclase and subsequent elevation of intracellular cyclic adenosine monophosphate (cAMP) levels. Increased cAMP then activates protein kinase A (PKA) and exchange protein activated by cAMP2 (EPAC2)/cAMP-guanine nucleotide exchange factor (GEF)II. Activation of PKA promotes closure of K_{ATP} channels and facilitates membrane depolarization. PKA also leads to inhibition of the delayed rectifying K^+ (Kv) channel, a negative regulator of insulin secretion in pancreatic β cells, resulting in prolongation of action potentials. Depolarization opens the voltage-gated Ca^{2+} channels (VDCC), allowing an increase of intracellular Ca^{2+} concentrations that mobilizes Ca^{2+} from intracellular stores through PKA- and EPAC2-dependent mechanisms. The increased Ca^{2+} concentrations eventually trigger fusion of insulin-containing granules with the plasma membrane and insulin secretion from the β cells. Increased Ca^{2+} levels also promote transcription of the proinsulin gene, thereby increasing the insulin content of the β cell. Activation of EPAC2 has been shown to increase the density of insulin-containing granules near the plasma membrane to potentiate insulin secretion from the β cell. ATP, adenosine triphosphate.

To date, little is known regarding the differences in signaling events downstream of GIPR and GLP-1R. Importantly, GLP-1 but not GIP stimulates glucose-dependent insulin secretion in perfused pancreatic islets from K_{ATP} channel-deficient (Kir6.2$^{-/-}$) mice[76]. Although the involvement of niflumic acid-sensitive ion channels has been shown in this process[77], further investigation is required to understand the potential diversity in the downstream mechanisms of GIPR and GLP-1R. Understanding the molecular mechanism underlying the remaining GLP-1 sensitivity in K_{ATP} channel-deficient mice should shed light on the selective inactivation of GIPR signaling in hyperglycemic conditions.

Another important aspect of the insulinotropic effects of GIP and GLP-1 is their synergy with the sulfornylurea drugs. Sulfornylureas efficiently cause mobilization of Ca^{2+} by closure of the K_{ATP} channels, membrane depolarization and subsequent VDCC opening, even in T2DM patients with impaired mitochondrial ATP production. As discussed earlier, GIP and GLP-1 affects events downstream of the K_{ATP} channel closure, thereby enhancing the ability of sulfornylureas to promote insulin secretion (Figure 5). In addition, it has recently been shown that certain sulfornylureas bind to EPAC2 and activate downstream molecules[78], thereby enhancing the insulinotropic effects of GIP and GLP-1. Taken together, it is clinically important to note that sulfornylureas and incretin-related therapies act synergistically to stimulate insulin secretion. Thus, caution is required for the combination of sulfornylureas and incretin-related drugs that potentially cause hypoglycemia, especially among Asians in whom diabetes is characterized by reduced insulin secretory capacity and sulfornylureas would be the first choice of drugs.

NON-INSULINOTROPIC FUNCTION OF GIP AND GLP-1 ON PANCREATIC β CELLS

Incretin was originally identified as the hormone that transmits signals from the gut to the pancreatic β cells, and the principal role of GIP and GLP-1 has generally been thought to stimulate insulin secretion. However, it has been shown that GIP and GLP-1 exert non-insulinotropic actions, such as controlling pancreatic β cell proliferation and survival.

For example, GIP has been shown to have an anti-apoptotic function in pancreatic β cells. This effect involves activation of the cAMP response element-binding (CREB) and Akt/PKB pathways (Figure 6). In INS-1 cells, binding of GIP to GIPR leads to the elevation of intracellular cAMP levels and activation of PKA, which then migrates into the nucleus and directly

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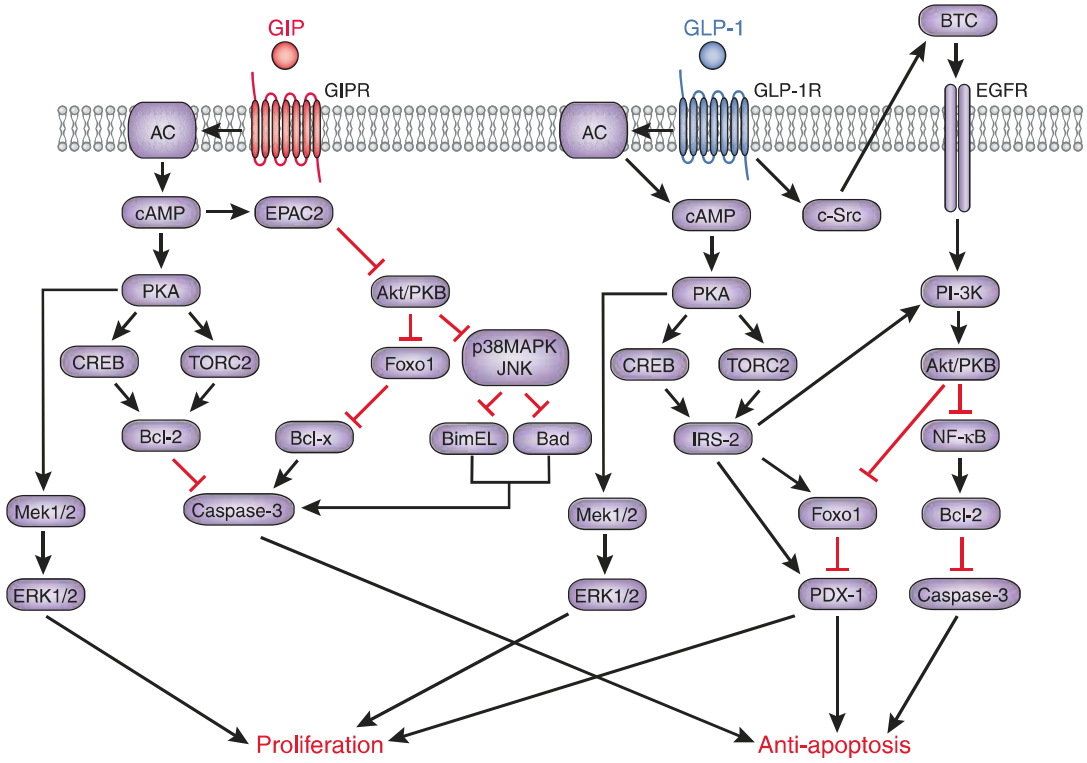

Figure 6 | Molecular mechanisms underlying the anti-apoptotic and proliferative effects of glucose-dependent insulinotropic polypepide (GIP) and glucagon-like peptide (GLP)-1. Signaling cascades linking the GIP receptor (GIPR) and the GLP-1 receptor (GLP-1R) with anti-apoptotic and proliferative effects share similarities and differences as shown. Involvement of epidermal growth factor (EGFR) and phosphoinositide 3-kinase (PI-3K) has been shown to be a critical difference between the GIPR- and GLP-1R-signaling pathways. AC, adenylate cyclase; Akt, v-akt murine thymoma viral oncogene homolog; Bad, Bcl-2 antagonist of cell death; Bcl, B-cell CLL/lymphoma; BimEL, Bcl-2 interacting mediator of cell death EL; BTC, betacellulin; cAMP, cyclic adenosine monophosphate; CREB, cAMP response element-binding; c-Src, proto-oncogene tyrosine-protein kinase Src; EPAC2, exchange protein directly activated by cAMP2; ERK, extracellular signal-regulated kinase; Foxo1, forkhead box protein O1; IRS-2, insulin receptor substrate 2; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; Mek, mitogen-activated protein kinase kinase; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; PDX-1, pancreas/duodenum homeobox protein 1; PKA, protein kinase A; PKB, protein kinase B; TORC2, transducer of regulated CREB activity 2.

phosphorylates nuclear CREB[79,80]. In addition, activated PKA inhibits AMPK, which then results in de-phosphorylation and nuclear import of the transducer of regulated CREB activity 2 (TORC2)[79]. In the nucleus, phosphorylated CREB and TORC2 form a complex in the promoter of the anti-apoptotic gene *bcl2*, thereby promoting its gene transcription[79]. Binding of GIP to GIPR also results in the activation of Akt/PKB, promoting phosphorylation of the nuclear transcription factor Foxo1 in INS-1 cells (Figure 6)[81]. Phosphorylated Foxo1 is exported from the nucleus, leading to downregulation of the pro-apoptotic gene *bax*, one of the Foxo1 direct target genes, which promotes β cell apoptosis in response to glucolipotoxicity[81]. Downregulation of Bax and upregulation of Bcl-2 is observed not only in INS-1 cells, but also in islets of the Vancouver diabetic fatty (VDF) Zucker rats receiving 2-week continuous infusion of GIP[81]. While the mechanisms underlying activation of Akt/PKB by GIP are yet unclear, it has been shown recently that activation of EPAC2, but not PI3-K, Erk1/2 or PKA, is responsible for Akt/PKB activation and the anti-apoptotic function of GIP[82]. Furthermore, activation of Akt/PKB by GIP has been shown to suppress mitochondrial translocation of Bad and BimEL and the subsequent activation of caspase-3 by inhibiting p38 MAPK and JNK in INS-1 cells exposed to staurosporin, a rapid activator of the mitochondria-mediated apoptotic pathway[83,84]. Suppression of p38 MAPK and JNK has also been shown to be critical for the anti-apoptotic actions of GIP in INS-1 cells exposed to endoplasmic reticulum (ER) stress and genotoxic stress[84].

The anti-apoptotic function of GLP-1 on pancreatic β cells was suggested by studies of the pancreas of exendin-4-treated *db/db* mice or GLP-1-infused diabetic VDF Zucker rats, which showed an increase in β cell mass and a decrease in apoptotic β cells[85,86]. Subsequently, it was shown that activation of GLP-1R by exendin-4 inhibits apoptosis of MIN6 cells exposed to hydrogen peroxide in a cAMP- and PI3K-dependent manner, in association with upregulation of Bcl-2 and Bcl-xL, and reduced poly-(ADP-ribose)-polymerase[87]. Activation of PKA and EPAC2 by elevated cAMP levels was also shown to inhibit activation of

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caspase-3 and subsequent apoptosis of palmitate-treated RINm5F cells[88] (Figure 6). Activation of Akt/PKB, a downstream effecter of PI3K, by GLP-1 was also shown to prevent apoptosis of INS-1 cells in response to glucolipotoxicity or staurosporin[89,90], possibly through the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) and the upregulation of the anti-apoptotic NFκB target genes *bcl2* and *IAP2*[89]. GLP-1R activation also reduces the ER stress response, thereby promoting β cell survival in INS-1 cells as well as in human islets[91–93]. A critical difference in the anti-apoptotic function of GLP-1 is the requirement for PI3K, which is not required for the anti-apoptotic action of GIP. While activation of Akt/PKB by GIP is mediated by EPAC2[82], activation of Akt/PKB by GLP-1 requires CREB-mediated induction of IRS-2, which might also potentiate PI3K and Akt/PKB[94] (Figure 6). The physiological impact of differences in PI3K requirement and other yet to be identified differences between GIP and GLP-1 have recently been investigated in mice treated with GLP-1R agonist, GIPR agonist or DPP-4 inhibitor[95]. This study showed that GLP-1R agonist exerts the most robust effect on the survival of β cells compared with that of GIPR agonist or DPP-4 inhibitor *in vivo* in mice[95]. Further investigation of the mechanisms underlying the effects of GIP and GLP-1 could show potential therapeutic targets to increase β cell mass by inhibiting apoptosis.

Another important aspect of GIP and GLP-1 action on β cells is the stimulation of the proliferation of β cells and/or progenitor cells. An early investigation evaluating the effects of exendin-4 in rats showed that activation of GLP-1R promotes proliferation and neogenesis of pancreatic β cells[96]. Later, proliferative effects were also shown of GIPR activation in INS-1 cells[97,98]. It has been shown that GIP activates the Raf-Mek1/2-ERK1/2 signaling module through cAMP/PKA signaling in GIPR-overexpressing Chinese hamster ovary cells[98–100] (Figure 6). Consistent with these observations, PKA and MEK inhibitors have been shown to prevent GIP-induced proliferation of cultured islet cells[101]. The same study further showed that GIP, as well as GLP-1, induces transcription of cyclin D1 that is critical for G1 phase progression and S-phase entry in most cell types[101]. Interestingly, PI3K inhibitor also prevents GIP and GLP-1-dependent proliferation of cultured islet cells and INS-1 cells, suggesting involvement of the PI3K pathway in the proliferation of pancreatic β cells induced not only by GLP-1, but also by GIP. Delineation of the proliferative action of GLP-1 further showed that it involves activation of PI3K and upregulation of PDX-1 transcription through transactivation of epidermal growth factor receptor (EGFR)[102,103]. Although these findings could explain the involvement of EGFR transactivation and subsequent PI3K activation in the proliferative action of GLP-1, whether or not the same mechanism might be involved in the proliferative action of GIP must be investigated. Importantly, the proliferative and anti-apoptotic effects of GIP and GLP-1 on pancreatic β cells could be achieved by pharmacological levels of incretins rather than physiological levels, because no reduction of β cell mass has been reported in animals lacking both GIPR and GLP-1R[25,27,29]. Nevertheless, the proliferative effects of GIP and GLP-1 on pancreatic β cells are clinically relevant for treatment of diabetes, and remain to be tested in patients with diabetes.

EFFECTS ON GLUCAGON SECRETION FROM PANCREATIC α CELLS

The effects of GLP-1 and GIP on glucagon secretion from pancreatic α cells are opposing. As early as the 1970s, infusion of GIP was shown to counteract suppression of glucagon secretion by glucose in rats or isolated rat islets[11,104]. Later, this was confirmed in healthy humans during euglycemic, but not during hyperglycemic, clamp studies[105], as well as in T2DM patients during meal-tolerance tests[106]. Furthermore, it has been shown that GIPR is expressed in human and mouse pancreatic α cells and that GIP stimulates glucagon secretion from cultured α cell-line αTC-1 cells with a concomitant increase in intracellular cAMP levels[106]. Although its physiological importance remains unknown, enhancement of glucagon secretion by GIP hinders clinical usage of GIP as a treatment for diabetes.

In contrast, GLP-1 has been shown to suppress glucagon secretion when plasma glucose levels are above fasting level[107]. This is clinically important because GLP-1 loses its inhibitory effect on glucagon secretion at hypoglycemic levels and does not attenuate the counter-regulatory responses to hypoglycemia. Furthermore, it has recently reported that insulin stimulation and glucagon inhibition contribute equally to the effect of GLP-1 on glucose turnover in T2DM patients[108]. Despite of its clinical importance, the mechanism underlying the suppression of glucagon secretion by GLP-1 remains unclear. While GLP-1R mRNA is detected in approximately 20% of cultured α cell-lines[109], expression of the GLP-1 receptor in pancreatic α cells *in vivo* is controversial, and there is no evidence that GLP-1 directly acts on α cells. How then does GLP-1 inhibit glucagon secretion? Glucagon secretion is strongly inhibited by GLP-1 in type 1 diabetes patients with no remaining insulin secretory capacity[110], suggesting that insulin, which is generally thought to suppress glucagon secretion, is not required in this process. Importantly, it has been shown that GLP-1 stimulates pancreatic somatostatin secretion[111] and that the inhibitory effect of GLP-1 on glucagon secretion is abolished by somatostatin antibodies and a somatostatin receptor 2 antagonist in isolated rat pancreas[112]. Although expression of the GLP-1R in δ cells also remains controversial, these studies suggest that the suppression of glucagon secretion by GLP-1 is mediated by somatostatin.

PHYSIOLOGICAL FUNCTION OF GIP IN FAT ACCUMULATION

Fats strongly enhance GIP secretion[38,113], and GIP levels are high in obese T2DM patients[114]. GIP has been proposed to have a physiological role on nutrient uptake into adipose tissues, thereby linking overnutrition to obesity. An initial clue came in the early 1980s from an experiment showing that GIP, in the

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presence of insulin, induces fatty acid incorporation into rat epididymal fat pads[115]. Later, GIPR was shown to be expressed in adipose tissues[116], and genetic ablation of GIPR further shows the critical role of GIP in fat accumulation[26].

High-fat diets are one of the well-known environmental determinants of obesity. Although control mice on a high-fat diet for 50 weeks show weight gain and a marked increase in visceral and subcutaneous fat mass and liver steatosis, such weight gain and adiposity was not observed in GIPR-deficient mice on the same high-fat diets[26]. GIPR-deficient mice on high-fat diets showed energy intake similar to that of control mice, but showed higher energy expenditure as revealed by a reduction of oxygen consumption and respiratory quotient during the light phase, the latter indicating that fat is utilized as the preferred energy substrate in GIPR-deficient mice. Furthermore, GIPR-deficient mice show increased adiponectin secretion, which promotes fat oxidation in muscle and increases the respiratory quotient[117,118]. In addition, genetic ablation of GIPR in obese *ob*/*ob* mice, in which a defect in the leptin gene results in hyperphagia and subsequent obesity[119], ameliorates not only obesity by increasing energy expenditure[26,53], but also insulin insensitivity and glucose tolerance without seriously affecting insulin secretion[54]. These observations were confirmed in high-fat fed mice and obese *ob*/*ob* mice treated with a GIPR antagonist, (Pro3)GIP[120–122] and in mice lacking GIP-secreting K cells[123], establishing the critical role of GIP in fat accumulation.

Although GIP was shown to increase the activity of lipoprotein lipase (LPL), an enzyme that is bound to the cell surface of adipocytes and hydrolyzes lipoprotein-associated triglycerides to produce free fatty acids available for local uptake[26], the molecular mechanism by which GIP acts on adipocytes is largely unknown. It was recently shown that binding of GIP to GIPR in 3T3-L1 cells and rat epididymal fat results in enhanced secretion of resistin through a pathway involving p38 MAPK and the stress-activated protein kinase/Jun amino-terminal kinase (SAPK/JNK)[124]. GIP activates PI3K and Akt/PKB through secreted resistin, thereby suppressing AMPK and increasing LPL activity in adipocytes[124,125]. Interestingly, another GIPR agonist, D-Ala^{2}GIP(1–30), shows a potency equivalent to GIP(1–42) on β cell function and survival, but greatly reduced action on lipoprotein lipase activity in 3T3-L1 cells[126]. Further investigation on the differential effects of D-Ala^{2}GIP(1–30) in β cell and adipocytes might shed light on the molecular mechanisms underlying GIP action in fat accumulation as well as might open up a possibility of GIP-based anti-diabetic therapy that does not promote obesity. Importantly, GLP-1 does not show any role in fat accumulation. While GLP-1R is expressed in adipocytes[127], activation of GLP-1R affects none of the aforementioned signaling molecules and does not increase LPL activity in adipocytes[124,125].

ENDOGENOUS GIP AND GLP-1 IN BONE METABOLISM

Regulation of bone metabolism is another important physiological function of GIP and GLP-1. A role of GIP in bone metabolism was first suggested by the presence of GIPR in bone and the suppression of ovariectomy-induced bone loss by GIP administration[128]. Later, the role of endogenous GIP in bone formation became evident in GIPR-deficient mice, which show thinner bone trabeculae reminiscent of osteoporosis[129]. Bone histomorphometrical analyses showed that bone formation parameters are significantly lower and the number of osteoclasts is significantly increased in GIPR-deficient mice, indicating high-turnover osteoporosis[129]. In addition, GIP suppresses apoptosis of osteoblasts *in vitro*, suggesting that GIP stimulates bone formation by inhibiting apoptosis of osteoblasts[129]. Enhancement of bone formation by GIP, through suppression of osteoclasts and prevention of osteoclast apoptosis, has also been reproduced in GIP transgenic mice[130–132]. Furthermore, GIP might facilitate calcium deposition in bone in response to meal ingestion because postprandial plasma calcium levels are enhanced in GIPR-deficient mice[128]. Although these findings imply that postmenopausal women might be osteoporosis-prone partly as a result of reduced GIP response[133], it remains to be determined whether GIP exerts an osteogenic function in humans.

A role of endogenous GLP-1 in bone metabolism was shown by detailed analyses of GLP-1R-deficient mice that showed cortical osteopenia and bone fragility in addition to increased osteoclastic numbers and bone resorption activity[134]. Unlike GIP, GLP-1 has no direct effect on osteoblasts and osteoclasts, and GLP-1 inhibits bone resorption indirectly through upregulation of calcitonin[134,135]. Although exendin-4 administered at pharmacological levels has been shown to promote bone formation in rats[136], whether GLP-1-based therapies show any effects on bone metabolism in human remains to be addressed in the future.

PHYSIOLOGICAL FUNCTIONS OF ENDOGENOUS GIP AND GLP-1 IN OTHER ORGANS

Receptors for GIP and GLP-1 are expressed in a wide variety of organs in addition to the pancreas, fats and bones. Within the brain, GIP is strongly expressed in neurons of the hippocampus, and the olfactory bulb and Purkinje cells of the cerebellum[137,138], and GIPR is expressed in several brain regions including the cerebral cortex, hippocampus and olfactory bulb[139,140]. Notably, expression of GIPR in neuronal progenitors of the dentate gyrus in the hippocampus suggests involvement of GIP in regulation of neurogenesis and memory formation[137]. Indeed, proliferation of neuronal progenitors is enhanced by infusion of GIP, and is decreased in the dentate gyrus of GIPR-deficient mice[137]. Consistent with the proliferative effects of GIP on neuronal progenitors, activation of GIPR by GIP analogue enhances LTP formation in hippocampal slice culture, whereas inhibition of GIPR by the GIP antagonist (Pro3)GIP reduces LTP[141]. GIP-transgenic (Tg) mice show improved performance in a memory-related behavioral task[142]. Similarly, GLP-1 enhances proliferation of neuronal progenitors[143,144] and has been shown to enhance LTP[145–147], and GLP-1R-deficient mice show impaired performance in memory-related behavioral tasks[145]. In addition, GLP-1 is protective against neuronal apoptosis in the

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Alzheimer's disease model[148,149]. Taken together, both GIP and GLP-1 could proliferate neuronal progenitors, thereby enhancing memory formation.

Another function of GIP in the brain is regulation of appetite and satiety. Ovariectomy-induced obesity has been prevented by GIPR deficiency, which could be explained partly by reduced expression of orexigenic neuropeptide Y (NPY) in the hypothalamus and subsequent reduction of food intake in the absence of GIPR[150]. Cerebral infusion of NPY stimulates neuronal secretion of GIP, implying that GIP acts as a negative regulator of NPY and can thereby control food intake[151]. However, careful consideration is required for anti-obesity function of GIP in the brain because GIP has its direct effect on adipose tissues. Future studies using brain-specific GIPR-deficient mice could clarify roles of GIP in the central nervous system. Regarding the regulation of appetite and satiety, both intracerebroventricular and peripheral infusion of GLP-1R agonists also inhibit food intake[152,153]. Further examination using the GLP-1 and GLP-1R antagonist exendin(9–39) confirms the inhibitory actions of endogenous GLP-1 on food intake[154,155]. GLP-1R is expressed in the arcuate nucleus and other hypothalamic regions involved in regulation of food intake[156], and destruction of the arcuate nucleus abolishes the inhibitory effect of GLP-1 on food intake[157]. These lines of evidence imply that not only GIP, but also GLP-1 controls food intake and satiety in humans.

In addition to these functions, GLP-1 is involved in gastrointestinal motility as well as in the reduction of cardiac contractility. Although GLP-1 inhibits gastric emptying[158,159], GIP has been shown to have little effect on gastric emptying in humans and mice[106,160]. GLP-1 action on the heart was also studied in GLP-1R-deficient mice that display increased left ventricle thickness, impaired left ventricle contractility and diastolic dysfunction[161]. In addition, GLP-1 administration in animal models or humans with cardiac injuries (e.g. acute myocardial infarction and dilated cardiomyopathy) significantly improves cardiac performance[162–166], suggesting not only beneficial effects of GLP-1 in heart diseases but also supporting the existence of physiological roles of GLP-1 on the heart. GIP action on the heart remains to be examined in the future.

INCRETIN-BASED THERAPIES FOR T2DM

Although endogenous GIP exerts strong insulinotropic effects in healthy subjects, the severe reduction in the insulinotropic effect of GIP[52,53] and the GIP-dependent enhancement of postprandial glucagon response[106] have discouraged development of GIP-based therapies for T2DM. In contrast, the insulinotropic effect of GLP-1 is substantially preserved in T2DM[53,167]. Long-term intravenous infusion of GLP-1 has been shown to improve glycemic control[168], establishing GLP-1 and GLP-1R signaling as attractive therapeutic targets for T2DM. Indeed, GLP-1R agonists (e.g. liraglutide and exenatide) and DPP-4 inhibitors (e.g. sitagliptin and vildagliptin) have been widely and successfully used. Furthermore, recent clinical data suggest that incretin-based therapies are more effective in Japanese patients compared with Caucasian patients[169–175]. The effectiveness of incretin-based therapies is consistent with the reduced early insulin secretory capacity in T2DM patients in Asia countries including Japan[176], and further suggests that such reduced early insulin secretory capacity could be partly due to their considerably lower levels of intact GLP-1, which has been recently revealed in Japanese subjects[42]. Incretin-based therapies have recently become widely available in Asian countries. However, their effectiveness in the regulation of long-term glycemic control, preservation of β cell mass and function, and the prevention of macro and microcomplications are not known and must be carefully followed for years. Nevertheless, given the pathophysiology of Asian T2DM (insulin deficiency rather than insulin resistance), incretin-based therapies that primarily correct impaired early insulin secretion might well be highly suitable in the treatment of Asian T2DM and have the potential to be a first choice therapy as is presently the case for metformin in Caucasian T2DM[177,178].

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