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REVIEW

Improved glucose regulation in type 2 diabetic patients with DPP-4 inhibitors: focus on alpha and beta cell function and lipid metabolism

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Abstract Inhibition of dipeptidyl peptidase-4 (DPP-4) is an established glucose-lowering strategy for the management of type 2 diabetes mellitus. DPP-4 inhibitors reduce both fasting and postprandial plasma glucose levels, resulting in reduced HbA_{1c} with low risk for hypoglycaemia and weight gain. They act primarily by preventing inactivation of the incretin hormones glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1, thereby prolonging the enhanced endogenous levels of these hormones after meal ingestion. This in turn causes islet and extrapancreatic effects, including increased glucose sensing in islet alpha and beta cells. These effects result in increased insulin secretion and decreased glucagon secretion being more effective in hyperglycaemic states and reduced insulin secretion and increased glucagon secretion being more effective during hypoglycaemia. Other secondary pharmacological actions of DPP-4 inhibitors include mobilisation and burning of fat during meals, decrease in fat extraction from the gut, reduction of fasting lipolysis and liver fat and increase in LDL particle size. These actions contribute to the clinical effects of DPP-4 inhibition, and the reduced demand for insulin could also lead to a durability benefit. This review summarises the current knowledge of the secondary pharmacological actions of DPP-4 inhibitors that lead to improved glucose regulation in patients with type 2 diabetes, focusing on alpha and beta cell function and lipid metabolism.

Keywords Dipeptidyl peptidase-4 inhibitors · GIP · GLP-1 · Glucagon · Insulin · Review

Abbreviations

Apo B-48	Apolipoprotein B-48
CV	Cardiovascular
DPP-4	Dipeptidyl peptidase-4
FPG	Fasting plasma glucose
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide-1
HGP	Hepatic glucose production
IFG	Impaired fasting glucose
ISR	Insulin secretory rate
Rd	Rate of disappearance of glucose
Si	Sensitivity index

Introduction

Islet dysfunction is a prerequisite for the development of type 2 diabetes mellitus and is characterised by inadequate insulin secretion in the face of increased demand for insulin in combination with augmented glucagon secretion in spite of hyperglycaemia [1]. The inadequate insulin secretion is caused by reduced sensitivity of the beta cells to glucose and a progressive decrease in insulin secretion capacity [2–5]; the augmented glucagon secretion is caused by its impaired suppression by glucose [6]. The reduced sensitivity of beta cells to glucose appears mainly to manifest itself in susceptible individuals with an inherently lower capacity for insulin secretion subsequent to increased demand for insulin because of greater triacylglycerol storage in non-fat tissues, termed lipotoxicity [4]. The demand for insulin is increased further by reduced glucose sensitivity in alpha cells, resulting in inappropriately

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elevated glucagon levels and, eventually, glucotoxicity through increased fasting plasma glucose (FPG) [7]. The capacity for insulin secretion then diminishes over time [1]. Type 2 diabetes is also associated with diminished glucagon counter-regulation in the face of hypoglycaemia [8]. These combined islet defects result in fasting and postprandial hyperglycaemia because of increased hepatic glucose production (HGP) and impaired glucose utilisation together with defective ability to counteract hypoglycaemia. Therefore, islet defects render type 2 diabetes a disorder in which there is deficient glucose regulation during both hyperglycaemia and hypoglycaemia. Hence, islet dysfunction is an important target for correcting glucose abnormalities in type 2 diabetes.

Until the advent of dipeptidyl peptidase-4 (DPP-4) inhibitors, there were no oral antihyperglycaemic agents for management of these multiple underlying pathologies. The initial premise for using DPP-4 inhibition as a glucose-lowering strategy was based on the effects of glucagon-like peptide-1 (GLP-1), and the potential of this treatment in type 2 diabetes was first reported in 1992 [9]. A few years later, it was demonstrated that GLP-1 is inactivated by DPP-4, suggesting that inhibition of DPP-4 may extend and enhance the physiological effects of GLP-1 [10]. This triggered the search for DPP-4 inhibitors that could be used therapeutically [11, 12]. A proof-of-concept study demonstrating improved glycaemia by DPP-4 inhibition in type 2 diabetes was published in 2002 [13] and the first DPP-4 inhibitor entered the market in 2006. Since then, at least five DPP-4 inhibitors have been licensed for the treatment of type 2 diabetes; they are all well tolerated, efficacious and easy to use [14]. These DPP-4 inhibitors differ in their chemical structures, metabolic pathways, pharmacokinetics and interactions with the DPP-4 catalytic site [14]. However, they all prevent the inactivation of GLP-1 and glucose-dependent insulinotropic polypeptide (GIP), prolonging meal-induced increases in the active forms of these two incretin hormones. We consider this to be the primary pharmacological action of the DPP-4 inhibitor class. The prolongation of meal-induced increases in GLP-1 and GIP in turn results in pancreatic and extrapancreatic effects, which we characterise as secondary pharmacological actions of DPP-4 inhibitors, which in turn results in the clinical benefits of reduced glycaemia without increased risk for hypoglycaemia or weight gain.

Here we review the secondary pharmacological actions of DPP-4 inhibitors on alpha and beta cell function and on lipid metabolism that lead to improved glucose regulation in patients with type 2 diabetes. We have not considered all potential benefits (e.g. on bone or immune function) nor have we addressed potential adverse effects of DPP-4 inhibitors, since these aspects have been covered in previous reviews [15–18]. Instead, our focus is to highlight the mechanism studies in humans that explain the clinical benefits of DPP-4 inhibitors.

Search strategy

We conducted a systematic search in PubMed using all synonyms of DPP-4 inhibitors and filtered all English-language journal articles restricted to human studies up until 10 June 2015. This comprised of a pool of 3,302 articles. First, from this pool, all articles having the keywords 'vildagliptin', 'sitagliptin', 'saxagliptin', 'alogliptin' or 'linagliptin' and 'patients', 'individuals' or 'subjects' in either the title or text of the abstract were shortlisted (N1 = 878). Second, from the pool, all articles having the keywords 'vildagliptin', 'sitagliptin', 'saxagliptin', 'alogliptin' or 'linagliptin' and 'study' in either the title or text of the abstract were shortlisted in parallel (N2 = 659). Duplicates were removed (N = 512) and the abstracts of the 1,025 resulting articles were then screened manually for relevance to beta cells, alpha cells and extrapancreatic effects on lipid metabolism secondary to prolongation of the meal-induced increases in GLP-1 and GIP (references to articles are not cited; this information is available from the authors on request). We did not consider papers that reported only homeostasis model assessment of beta cell function and homeostasis model assessment insulin resistance as measures of beta cell function and insulin resistance since the number of relevant papers would greatly exceed the allowed number of references in this review.

Effects on beta cells

Analysis of insulin or C-peptide levels after ingestion of glucose or a mixed meal is one of the most common methods used to explore the effects of DPP-4 inhibitors on beta cells. In such studies, however, the data need to be adjusted for the prevailing glucose levels because it is the relationship between insulin secretion and glucose concentration that is the most basic reflection of beta cell function and not the actual levels of insulin or C-peptide. When analyses were adjusted accordingly, all DPP-4 inhibitors were shown to increase in insulin and C-peptide relative to glucose levels after oral glucose or mixed-meal administration [19–25]. Some studies in which an insulinogenic index has been calculated (insulin or C-peptide level divided by glucose level) have shown that DPP-4 inhibitors do indeed increase beta cell function [13, 22, 26–29]. Nevertheless, the extent of this stimulation is difficult to establish because glucose levels are reduced by DPP-4 inhibition. Instead, beta cell function is better assessed after i.v. administration of glucose. Improvements in acute insulin response to glucose given by the i.v. route have been seen in individuals with impaired fasting glucose (IFG) following treatment with vildagliptin [30], but not sitagliptin [31], and in patients with type 2 diabetes following treatment with vildagliptin and sitagliptin [26, 32–35]. This suggests that DPP-4 inhibitors can improve beta cell function both within

and outside the context of meal ingestion. In line with this, vildagliptin and sitagliptin improved insulin secretion in response to stimulation with glucose given orally and intravenously; paradoxically no change in the incretin effect was observed [33, 35].

An even more appropriate estimation of beta cell function is provided by calculating the insulin secretory rate (ISR). This is performed by deconvolution of C-peptide levels (C-peptide is co-secreted with insulin and has linear clearance that is easily estimated) and the AUC for ISR or glucose (insulin secretion relative to glucose [ISR/G]) can then be used as an index of beta cell function. This index was consistently found to be increased after a mixed meal in vildagliptin-treated individuals who had impaired glucose tolerance [36], IFG [30] or type 2 diabetes and who received vildagliptin as monotherapy, either acutely [37] or after 2 years [38], or who received vildagliptin as add-on to metformin [39] or sulfonylureas [40]. It is reasonable to assume that other DPP-4 inhibitors have the same effect. Furthermore, after a single dose of vildagliptin was given to patients with type 2 diabetes before their evening meal, ISR/G was increased significantly throughout the entire overnight post-absorptive period [37]. In these patients the effect was the same during the evening meal as before breakfast, suggesting that the raised ISR/G was not due to the increase in GLP-1 levels beyond those seen at the beginning of meals physiologically, but rather due to an extension of the physiological effect over the entire overnight period [37].

Another measure for estimating insulin secretion is to calculate the ratio of the incremental AUC for C-peptide to the AUC for glucose during standard meal tests. Using this index in a study of vildagliptin in metformin-treated patients with inadequate glycaemic control, insulin secretion was found to be increased by >30% after 12 weeks of vildagliptin treatment, and this was sustained throughout 1 year of treatment [19]. In another study, beta cell function assessed as ISR/G during meal tests was sustained over 2 years with vildagliptin in patients with type 2 diabetes and mild hyperglycaemia, but not in the placebo comparator group [38]. Improved beta cell function also appeared to be maintained over a 2 year period with sitagliptin plus metformin treatment [41].

The relationship between insulin secretion and glucose concentrations includes the two components of sensitivity and capacity (Fig. 1). A more complete measure of beta cell function therefore is needed to analyse both these components. The sensitivity of insulin secretion to glucose is a particularly important measure since it is an early indicator of defect in beta cell function in type 2 diabetes. This measure can be estimated by the Mari model which estimates the ISR at different glucose levels and therefore provides the full-range sensitivity of glucose to stimulate insulin secretion [42–44]. When this model was applied to data from a study in drug-naive patients with type 2 diabetes and mild hyperglycaemia, vildagliptin increased glucose sensitivity of insulin secretion

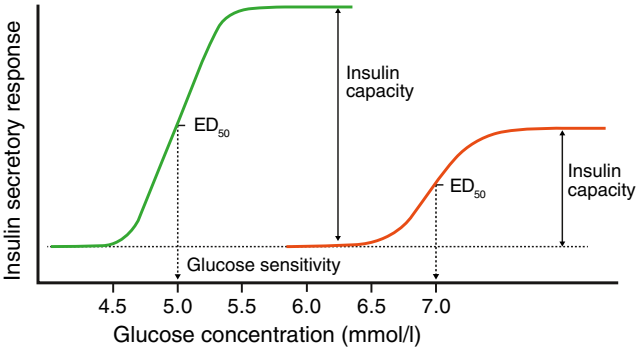

Fig. 1 Schematic illustration of the relationship between insulin secretion and glucose concentration. Glucose sensitivity reflects the relationship between insulin secretion and glucose concentration and is quantified by the ED_{50} (glucose concentration eliciting 50% of maximal insulin secretion [insulin capacity]). The two curves represent the relationship under normal conditions (left) and in type 2 diabetes mellitus (right), illustrating that type 2 diabetes is characterised by reduced glucose sensitivity (increased ED_{50}) and reduced insulin capacity

but did not influence the glucose-insensitive stimulation of insulin secretion [42, 43]. Hence, according to this model, DPP-4 inhibitors seem to augment beta cell function mainly by increasing glucose sensitivity, with a glucose threshold of approximately 6 mmol/l. This has also been demonstrated in studies where a single dose of vildagliptin or sitagliptin increased plasma insulin levels when given before a 75 g OGTT in patients with type 2 diabetes [20, 23]; no such effect was found in healthy volunteers with an FPG of ≈4.5 mmol/l [45] or in individuals with IFG with an FPG of ≈5.5 mmol/l [31] (i.e. below the glucose threshold). These findings were extended by studies showing that sitagliptin improved beta cell glucose sensitivity during both a mixed meal and i.v. administration of isoglycaemic glucose in patients with type 2 diabetes [33, 46]. This implies, as has been shown with chronic sitagliptin or vildagliptin therapy, that DPP-4 inhibition specifically improves glucose sensing in beta cells regardless of the stimulus; this in turn may explain why there is no change in the incretin effect, which is calculated as the ratio of insulin secretion after oral vs i.v. administration of glucose at matching glucose levels [33, 35]. A most interesting observation made in patients on insulin therapy was that an improved glucose sensing was observed with sitagliptin, when evaluated by the C-peptide minimum model [21, 47], suggesting that DPP-4 inhibition increases glucose sensitivity of insulin secretion in patients on insulin therapy. Similarly, in a 16 week study in individuals with recent-onset type 2 diabetes, using the Mari model showed that a combination of alogliptin with pioglitazone increased the glucose sensing of insulin secretion whereas alogliptin as monotherapy had no effect on beta cell function [48]. Whether the finding that alogliptin alone did not increase beta cell glucose sensitivity in this study compared with the effects of other DPP-4 inhibitors in other studies reflects different effects of the different inhibitors remains to be explored in head-to-head studies. This result would

however suggest that thiazolidinediones may enhance the beta cell action of DPP-4 inhibition, which is an interesting observation requiring further study.

Beta cell function can also be measured by the total capacity to secrete insulin [49]. This may be related to beta cell mass, which is reduced in animal models of type 2 diabetes. GLP-1 and DPP-4 inhibition increase beta cell mass via increased beta cell neogenesis and decreased beta cell apoptosis when determined in models of diabetes using young animals, whereas this effect is not seen in more mature animals [50]. This suggests that treatment does not increase beta cell mass in older animals. Support comes from human data on the capacity of beta cells to stimulate insulin secretion as studied after combined glucose and arginine-stimulated C-peptide secretion rate. Thus, whereas there indeed was an increase in the capacity for insulin secretion after 3 or 12 months of treatment with vildagliptin [5, 32] or sitagliptin [34] as long as treatment was ongoing, such an effect was not maintained after a 3 month washout period. Similarly, a pilot study failed to show any evidence that sitagliptin preserved beta cell function after intensive insulin therapy [51]. These results together indicate that DPP-4 inhibition has no disease-modifying effect on beta cells up to at least 2 years of therapy and that the improved beta cell function is evident only as long as the inhibitor stimulates beta cell function. This failure is perhaps the greatest unrealised early promise of the GLP-1-based therapies. Although these data suggest that DPP-4 inhibition does not appear to restore beta cell mass, they are consistent with its maintenance of beta cell functional mass, possibly due to the long-term influence of GLP-1 in association with reduced glycaemia and relieved glucotoxicity.

It is thus clear that DPP-4 inhibition stimulates insulin secretion in a glucose-dependent manner by increasing the glucose sensitivity of beta cells. This infers that the inhibition of insulin secretion is augmented when glucose levels fall to hypoglycaemic levels. This concept was examined in a study in drug-naive individuals with type 2 diabetes [52]. It was found that the inhibition of insulin secretion, as estimated by modelling of C-peptide data, was indeed augmented by vildagliptin when glucose levels were reduced to 2.5 mmol/l by means of a hyperinsulinaemic–hypoglycaemic clamp. This suggests that DPP-4 inhibition increases glucose sensitivity in beta cells over a large range of glucose levels, from hypoglycaemic to hyperglycaemic levels. As a consequence this would allow beta cells to adapt to the degree of insulin resistance, contributing to improved fasting and postprandial glycaemia [20, 22, 35] and the rapid increase or decrease in insulin secretion according to need enables the beta cells to show a better response to the next hyperglycaemic challenge. By extending the recovery time after a reduction in circulating glucose, allowing proinsulin to split into insulin and C-peptide due to improved proinsulin processing, DPP-4 inhibitors decrease the overall insulin exposure and the proinsulin-to-insulin ratio [20, 28, 53, 54].

Effects on alpha cells

Inhibition of glucagon secretion is an important target in the treatment of type 2 diabetes because hyperglucagonaemia contributes to both fasting and postprandial hyperglycaemia [55, 56]. It is therefore of great interest that DPP-4 inhibitors inhibit glucagon secretion in individuals with type 2 diabetes [11]. This was initially demonstrated in 2004 when vildagliptin was shown to inhibit the glucagon response to a mixed meal [56] and was later confirmed for vildagliptin, sitagliptin, saxagliptin and alogliptin in several studies [20, 30, 37, 42, 46, 48, 57–60]. DPP-4 inhibition has also been found to suppress the inappropriate glucagon response to oral glucose in patients with type 2 diabetes [20, 23, 25, 35]. One study demonstrated that the ability of insulin to reduce glucagon after meals was augmented by vildagliptin [59], thus revealing the additive glucagonostatic effects of insulin and DPP-4 inhibition. Another study showed that saxagliptin reduced the meal-induced increase in glucagon levels obtained by the sodium–glucose transport protein-2 inhibitor dapagliflozin [61]. In contrast, DPP-4 inhibition seems to have no effect on glucagon levels either in healthy individuals with a mean FPG of approximately 4.5 mmol/l [45] or in individuals with IFG and a mean FPG of approximately 6.0 mmol/l [31]. This shows a clear glucose-dependent mechanism for the inhibitory action of DPP-4 inhibition on glucagon secretion. A long-term study with vildagliptin as add-on to metformin treatment found that postprandial glucagon levels were still reduced after 2 years [62], demonstrating that the glucagonostatic effect of DPP-4 inhibition persists over time.

The inhibitory effect of DPP-4 inhibition on glucagon secretion is most likely explained by GLP-1, which is known to inhibit glucagon secretion in patients with type 2 diabetes [9]. The inhibitory effect of GLP-1 on glucagon secretion was found to be glucose dependent [63, 64] and this has been explained by increased sensitivity of glucose to inhibit glucagon secretion [6]. However, it is unlikely that GLP-1 has a direct effect on alpha cells, as GLP-1 receptors on pancreatic alpha cells are scarce or nonexistent [11]. Instead, secondary mechanisms may explain how GLP-1 mediates the inhibition of glucagon secretion by DPP-4 inhibition. One such mechanism may involve increased intra-islet insulin concentrations since GLP-1 increases insulin secretion and insulin inhibits glucagon secretion [65]. To examine this possibility, insulinopenic patients with type 1 diabetes have been studied based on the assumption that: if DPP-4 inhibition reduces glucagon by increasing intra-islet insulin, there would be no glucagonostatic effect in such patients. However, several studies have shown that indeed GLP-1 or DPP-4 inhibition reduces glucagon levels also in type 1 diabetes. Thus, in 1992 GLP-1 was shown to inhibit glucagon secretion in type 1 diabetes [9], and DPP-4 inhibition was found to suppress inappropriate glucagon secretion during mixed meals in type

1 diabetes to the same extent as seen in patients with type 2 diabetes [11, 66, 67]. This indicates that the suppression of inappropriate glucagon secretion by DPP-4 inhibition is not secondary to increased insulin secretion. An animal study has suggested that the glucagonostatic effect of GLP-1 is mediated by a local paracrine effect of somatostatin, stimulated by GLP-1, acting on the somatostatin receptor subtype 2 on alpha cells [68]. Studies on the detailed mechanism in humans remain to be performed.

In contrast to the suppressive effect of DPP-4 inhibitors on inappropriate glucagon secretion in response to glucose or mixed meals in type 2 diabetes, DPP-4 inhibition does not inhibit the glucagon counter-regulation to hypoglycaemia. On the contrary, one study in drug-naive individuals with type 2 diabetes demonstrated that DPP-4 inhibition by vildagliptin enhances alpha cell responsiveness to the stimulatory effect of hypoglycaemia [52], and sustained glucagon counter-regulation to hypoglycaemia has been shown for vildagliptin in insulin-treated patients with type 2 diabetes [69] and in patients with type 1 diabetes [67]. This demonstrates that DPP-4 inhibition has a dual effect on glucagon secretion such that when glucose levels are elevated glucagon levels are reduced whereas during hypoglycaemia glucagon levels are increased [11]. There are several mechanisms that may explain the counter-regulatory glucagon response to hypoglycaemia, as illustrated in Fig. 2. While it is known that hypoglycaemia per se reduces intra-islet insulin levels, adrenaline (epinephrine) released from the adrenal glands and autonomic nerves may contribute to glucagon counter-regulation. A potential additional mechanism for DPP-4 inhibition may be the incretin hormone GIP, which is known to stimulate glucagon release during euglycaemia [64] or hypoglycaemia [70], whereas it does not seem to affect glucagon secretion at elevated glucose levels. Hence, GIP has an effect on glucagon secretion that is in contrast to the effect of GLP-1. This suggests that the increase in GIP levels by DPP-4 inhibition may be the mechanism by which glucagon counter-regulation to hypoglycaemia is sustained or augmented by these drugs. Support for this comes from animal studies showing that mice with genetic deletion of the GIP receptors have a poorer glucagon counter-regulation to hypoglycaemia during vildagliptin treatment compared with normal mice with intact GIP receptors [71]. Therefore, although the reduction of glucagon during hyperglycaemia seems to be mediated by GLP-1, stimulation by DPP-4 inhibition of glucagon secretion during hypoglycaemia may be mediated by GIP. Together this suggests an important dual effect of DPP-4 inhibition on glucagon secretion mediated by both GLP-1 and GIP (illustrated in Fig. 3). Other factors may, however, also contribute: neural influences have been demonstrated in a study on sitagliptin in mice [72] and, furthermore, HGP is not only governed by the absolute portal glucagon levels but also by the portal glucagon: insulin ratio [73].

The dual effect that DPP-4 inhibition exerts on glucagon secretion may be of clinical importance. First, the GLP-1-mediated reduction in glucagon at elevated glucose levels may result in reduced HGP. This is supported by reports of a reduced postprandial HGP, associated with reduced glucagon levels, after treatment with vildagliptin [37] and also after sitagliptin in combination with metformin, although in the latter study sitagliptin alone had no effect [74]. In contrast, during euglycaemic–hyperinsulinaemic clamp after an overnight fast, when glucagon most likely is not alerted,

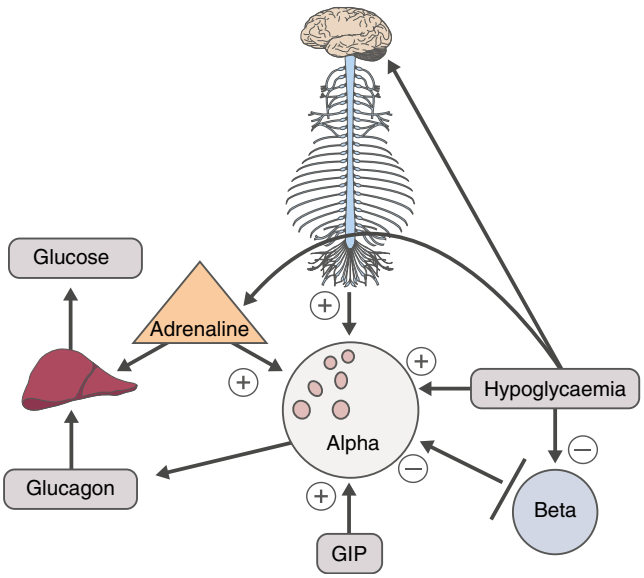

Fig. 2 Five main mechanisms underlying stimulation of glucagon secretion by alpha cells during hypoglycaemia: reduced beta cell secretion elicits a reduction in intra-islet insulin-dependent inhibition of glucagon secretion; direct effect of low glucose; activation of the autonomic nerves; stimulated release of adrenaline from the adrenals and action of GIP. The released glucagon and adrenaline then stimulate HGP resulting in the glucose counter-regulation

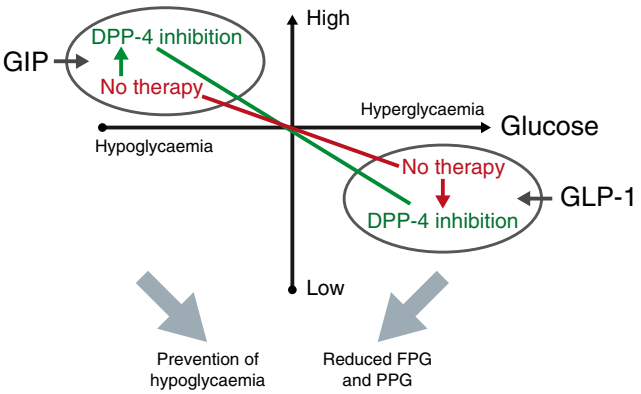

Fig. 3 Relationship between circulating glucose (*x*-axis: from hypoglycaemia through euglycaemia to hyperglycaemia) and glucagon secretion (*y*-axis from low to high levels) under normal conditions and during DPP-4 inhibition. DPP-4 inhibition reduces glucagon secretion at high glucose levels (through GLP-1) and augments glucagon secretion at low glucose levels (through GIP). These effects result in reduced FPG and postprandial plasma glucose (PPG) and prevention of hypoglycaemia

vildagliptin was found not to affect HGP in patients with type 2 diabetes [75]. The importance of the reduction in glucagon for the glycaemic effect of DPP-4 inhibition is also supported by the significant correlation between reduction of postprandial glucagon and postprandial glucose after treatment with vildagliptin in type 2 diabetes [56].

Second, the improved counter-regulation may contribute to the low risk for hypoglycaemia during treatment with DPP-4 inhibitors particularly if the DPP-4 inhibition is combined with insulin, due to sustained glucagon secretion [76]. Taken together, the dual effects of DPP-4 inhibition on glucagon secretion contribute to two of the main effects of this treatment—lowering of hyperglycaemia and protection from hypoglycaemia.

Intra-islet effects

There is accumulating evidence that DPP-4 is expressed in the pancreatic islets and in humans the expression seems mainly to be localised to alpha cells [77]. Also GLP-1 is expressed in islet alpha cells [78]. It is therefore possible that DPP-4 inhibition acts locally in the islets to protect the intra-islet inactivation of GLP-1, resulting in improved islet function, although there is a potential conflict in reported data since DPP-4 activity has been shown to positively correlate with insulin secretion in human islets [79]. Nevertheless, there is a possibility that DPP-4 inhibition may improve islet function through a local action to prevent local production of GLP-1. Support for this comes from studies showing that vildagliptin significantly potentiates glucose-stimulated insulin secretion in human islets [79] and that linagliptin improves beta cell function, provides protection against glucotoxicity, lipotoxicity and cytokine toxicity and stabilises active GLP-1 secreted from human islets [80].

Extrapancreatic effects on lipid metabolism

Vildagliptin has been shown to increase postprandial lipolysis in adipose tissue and increase postprandial fat oxidation in muscle, suggesting that the fat accumulated in adipocytes during fasting is mobilised and burned in muscle in the fed state during DPP-4 inhibition [81]. Furthermore, postprandial triacylglycerol-rich lipoprotein levels are reduced by DPP-4 inhibition with vildagliptin, sitagliptin and alogliptin [82–84]. A reduction in total serum triacylglycerol and chylomicrons was observed, reflecting reductions in chylomicron apolipoprotein B-48 (Apo B-48) and chylomicron cholesterol. The effect on Apo B-48 was later shown not to be due to improved glucose control with sitagliptin [85]. More recently it was shown that a single oral dose of sitagliptin reduces the production of intestinally derived Apo B-48-containing lipoprotein particles in healthy men [86]. Furthermore, fractional clearance of particles of both intestinal and hepatic origin and production of particles of hepatic origin were not affected. As chylomicrons are the initial lipoproteins into which dietary triacylglycerols are packaged, these findings suggest that DPP-4 inhibitors may have an inhibitory effect on fat absorption from the gut [87]. The effect of DPP-4 inhibitors on Apo B-48 production is probably mediated by GLP-1 receptors, as similar findings have been reported with a GLP-1 receptor agonist [88]. Further analysis of the original vildagliptin study demonstrated a decrease in postprandial remnant-like particle triacylglycerols and remnant-like particle cholesterol in type 2 diabetes, with a concomitant increase in LDL particle-size therapy, suggesting that GLP-1-based therapies may have unique mechanisms of relevance for cardiovascular (CV) risk [89].

In contrast to the effect vildagliptin has of mobilising fat during meals, vildagliptin was found to decrease the rate of fasting lipolysis as indicated by a reduction in palmitate flux [58]. As vildagliptin does not increase fasting insulin levels and as, in animal studies, both GLP-1 and GIP inhibit lipolysis, it is likely that this effect of vildagliptin is a direct incretin hormone-mediated extrapancreatic effect [11]. An inappropriate rate of fasting lipolysis is the major cause of elevated liver triacylglycerol concentrations; a reduction in lipolysis is thus expected to decrease liver triacylglycerol levels [90]. A substantial reduction in fasting liver triacylglycerol levels almost to levels of the non-diabetic control group was observed in a recently reported study with vildagliptin; this decrease was not due to the relationship between change in body weight and liver fat [75].

Integrating the secondary pharmacological effects with the clinical benefits of DPP-4 inhibitors

Beyond the primary pharmacology of preventing inactivation of GLP-1 and GIP to prolong their increased levels after meal ingestion, DPP-4 inhibition results in a number of secondary pharmacological effects, which are both pancreatic and extrapancreatic (Fig. 4). These effects result in reduction of postprandial plasma glucose and FPG levels, leading to reduced HbA_{1c} levels, with no increase in the risk for hypoglycaemia or weight gain. Increasing insulin secretion and decreasing glucagon secretion more effectively in hyperglycaemic states are likely the primary benefits of this class of drug, and the effects of GLP-1 on alpha and beta cells appear to be equally important for its glucose-lowering action [64]. An important effect of GLP-1 receptor agonists is a direct action on the brain stem to induce satiety and inhibit gastric emptying [59]. A variety of methods have been used to assess appetite and gastric emptying directly, including rate of ingested glucose into the circulation, surrogates of appetite

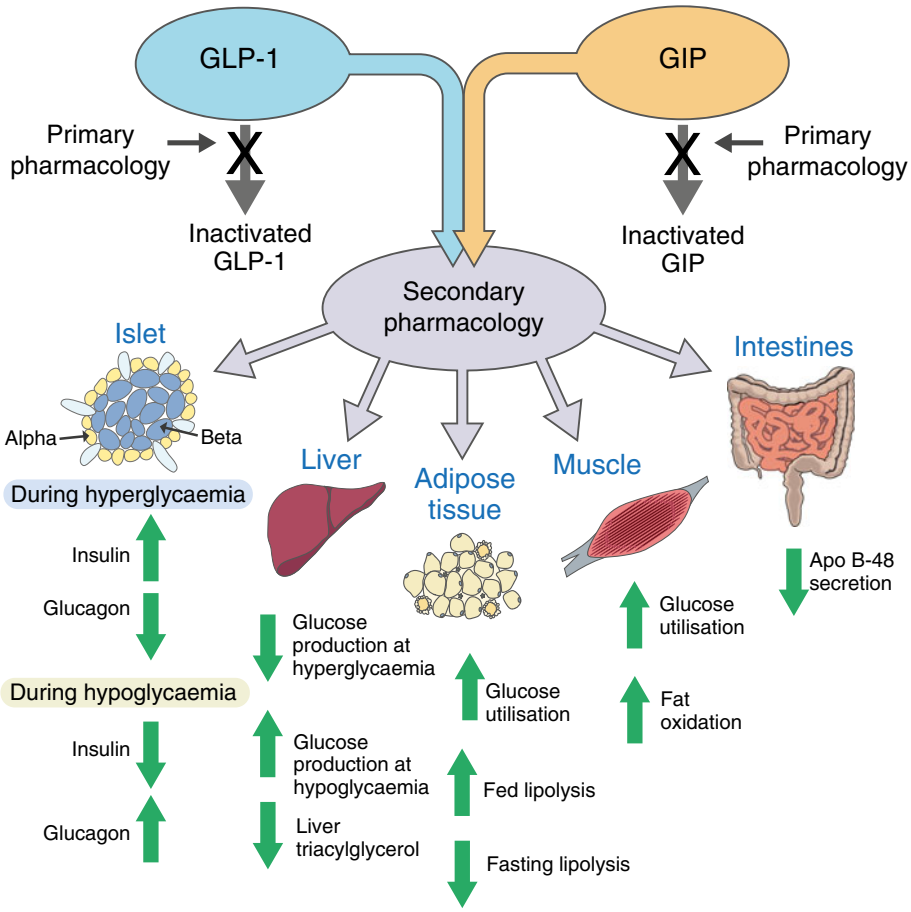

Fig. 4 The pharmacology of DPP-4 inhibitors is divided into primary pharmacology, which prevents the inactivation of GLP-1 and GIP, which prolongs the increase in active incretin hormones after meal ingestion, and secondary pharmacology, which involves several mechanisms in islets, liver, fat tissue, muscle and gut. The mechanisms involved in the secondary pharmacology explain the clinical effects seen during treatment with DPP-4 inhibitors

such as ghrelin and gastrin levels or the paracetamol test of gastric emptying and findings indicate that DPP-4 inhibitors have no effect on satiety and gastric emptying [59, 60, 91–95]. The prevailing evidence suggests that the effect of GLP-1 on satiety and gastric emptying requires doses of GLP-1 that are much higher than those achieved by DPP-4 inhibition [94]. Conversely, the reduced insulin secretion and increased glucagon levels in hypoglycaemia brought about by DPP-4 inhibition lead to better counter-regulation. This largely explains the low potential for hypoglycaemia by this class of drugs.

The effects of DPP-4 inhibitors on lipid metabolism all predict reduced CV risk. For instance, inhibiting lipolysis in the fasting state over many weeks should redistribute fat storage from non-fat tissues to the fat cells; the observation that there is reduced liver fat is consistent with this mechanism. On the other hand, mobilising and burning fat during meals avoids the accumulation of fat that could otherwise lead to weight gain and consequent CV risk. Furthermore, the reduced Apo B-48 level is consistent with reduced CV risk. However, despite reduced hyperglycaemia without increased hypoglycaemia as well as the improved lipid profile there has been no evidence of reduced CV risk when DPP-4 inhibitors have been added to other therapy in patients with type 2 diabetes and established CV risk over a median period of 3 years, as demonstrated for sitagliptin [96]. Of course it is possible that a primary prevention trial of longer duration might result in a CV benefit due to the beneficial effects on glycaemia and lipids.

As discussed in the introduction, insulin resistance can be due to glucose toxicity, due to inappropriately elevated glucagon levels and to lipotoxicity. Vildagliptin, sitagliptin and saxagliptin all improve a dynamic marker of insulin sensitivity, the oral glucose insulin sensitivity index (OGIS) [19, 24, 25, 46], likely due to a combination of reduced glucagon, reduced lipotoxicity and reduced glucotoxicity. The sensitivity index (Si) from an oral minimum model does not include the glucagon component and it is not predicted to have a lipotoxicity component after short duration. Inexplicably, after 9 days of vildagliptin treatment in patients with type 2 diabetes, there was no improvement in Si [54], whereas after 2 weeks there was a clear improvement in the Si in individuals with IFG [30].

There are a number of studies of DPP-4 inhibitors in which glucose utilisation has been assessed by a euglycaemic–hyperinsulinaemic clamp; the insulin concentrations used in these studies preclude any contribution of glucagon during the clamp. Glycogen storage was presumed to be the rate-limiting step in all of these clamp studies, except in one in which the

duration was more than twice that of the others [58]. This study revealed final insulin concentrations that were twice those found in the other clamp studies in which glucose utilisation in muscle was the presumed rate-limiting step [97]. We recalculated the data presented in that paper and found that the amount of glucose utilised during the clamp exceeded the amount of glycogen that could be stored. Since any glucose that cannot be oxidised or stored as glycogen must be converted to fat, we presume that lipogenesis is the rate-limiting step under this condition. Vildagliptin increased glucose utilisation in this clamp where thus we presume lipogenesis is the rate-limiting step.

Sitagliptin and vildagliptin also increased glucose utilisation in clamps where glucose utilisation in muscle was predicted to be the rate-limiting step [46, 98–100]. In contrast, vildagliptin did not increase glucose utilisation in two other clamp studies where glucose utilisation in muscle was predicted to be the rate-limiting step [5, 75]. Participants in both of these studies had lower baseline FPG values than the participants in any of the other studies, suggesting that the degree of glucotoxicity is a confounding variable in the response to a DPP-4 inhibitor [75]. In one of these studies, where there was no effect on glucose utilisation during the clamp, there was no change in fasting HGP, while FPG was reduced [75]; thus it follows that the fasting rate of disappearance of glucose (Rd) increased. The lack of effect on the clamp is not consistent with increased glycogen storage in muscle, suggesting that there could be increased lipogenesis. There was also a 25% reduction in liver fat [75].

Of course, increased lipogenesis would increase liver fat [101]; thus this hypothesis presumes that the reduction in lipolysis leading to reduced liver fat outweighs the increase in lipogenesis leading to increased liver fat. We believe this hypothesis merits further discussion and investigation.

The low hypoglycaemic potential of DPP-4 inhibitors prevents the weight gain associated with defensive eating to avoid hypoglycaemia. Thus, DPP-4 inhibitors do not lead to weight gain despite the caloric penalty associated with reducing glucose levels from above to below the renal threshold [102]. Although data is lacking for any direct mechanism that can explain this weight mitigation, one potential mechanism is the mobilisation and burning of fat during meals and another is the reduction in Apo B-48 secretion leading to decreased fat extraction from the gut. In contrast to GLP-1 receptor agonists, there is no satiety effect with DPP-4 inhibitors [91].

The lingering question is: are all of the secondary pharmacological effects presented here DPP-4 class effects? Some are clearly class effects as demonstrated by their being seen with more than one DPP-4 inhibitor. Some of these effects are only seen with one DPP-4 inhibitor and determining whether they are class effects awaits further studies. However, it is difficult to imagine why any of the meal-related effects would not be class effects. Therefore, DPP-4 inhibitors take advantage of both GLP-1 and GIP for their secondary pharmacology, which is mainly directed toward improvement of islet function. Since this class of drugs improve both beta and alpha cell dysfunction during both hyper- and hypoglycaemia, it represents the first class of drugs to target all of the combined manifestations of islet dysfunction in type 2 diabetes.

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Compliance with ethical standards

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Duality of interest BA has consulted for Novartis, GlaxoSmithKline, Merck, Sanofi, Novo Nordisk, Boehringer Ingelheim and Takeda, and has received lecture fees from Novartis, Merck, Novo Nordisk and Sanofi, all of which manufacture DPP-4 inhibitors or GLP-1 receptor agonists. JEF is employed by and own shares in Novartis Pharmaceuticals Corporation.

Contribution statement JEF designed the search strategy and conducted the abstract screening, analysis and interpretation. BA analysed and interpreted the screened articles. Both the authors had full access to all data and take responsibility for the integrity of the data and accuracy of analyses. Both the authors drafted the manuscript, read and approved the final draft.

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