

Incretins and other peptides in the treatment of diabetes

J. F. Todd and S. R. Bloom

Department of Metabolic Medicine, Imperial College London, Hammersmith Hospital, London, UK

Accepted 15 October 2006

Abstract

Glucagon-like peptide-1 (7-36) amide (GLP-1) is a gut hormone, released postprandially, which stimulates insulin secretion and insulin gene expression as well as pancreatic B-cell growth. Together with glucose-dependent insulinotropic polypeptide (GIP), it is responsible for the incretin effect which is the augmentation of insulin secretion following oral administration of glucose. Patients with Type 2 diabetes have greatly impaired or absent incretin-mediated insulin secretion which is mainly as a result of decreased secretion of GLP-1. However, the insulinotropic action of GLP-1 is preserved in patients with Type 2 diabetes, and this has encouraged attempts to treat Type 2 diabetic patients with GLP-1. GLP-1 also possesses a number of potential advantages over existing agents for the treatment of Type 2 diabetes. In addition to stimulating insulin secretion and promoting pancreatic B-cell mass, GLP-1 suppresses glucagon secretion, delays gastric emptying and inhibits food intake. Continuous intravenous and subcutaneous administration significantly improves glycaemic control and causes reductions in both HbA_{1c} and body weight. However, GLP-1 is metabolized extremely rapidly in the circulation by the enzyme dipeptidyl peptidase IV (DPP-IV). This is the probable explanation for the short-lived effect of single doses of native GLP-1, making it an unlikely glucose-lowering agent. The DPP-IV resistant analogue, exenatide, has Food and Drug Administration (FDA) approval for the treatment of Type 2 diabetes and selective DPP-IV inhibitors are under development. Both approaches have demonstrated remarkable efficacy in animal models and human clinical studies. Both are well tolerated and appear to have advantages over current therapies for Type 2 diabetes, particularly in terms of the effects on pancreatic B-cell restoration and potential weight loss.

Diabet. Med. 24, 223–232 (2007)

Keywords glucagon-like peptide-1, glucose-dependent insulinotropic polypeptide, incretin effect, Type 2 diabetes

Abbreviations DPP-IV, dipeptidyl peptidase IV; FDA, Food and Drug Administration; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1 (7-36) amide; NEP, neutral endopeptidase

The incretin effect

It has been demonstrated that, for a given rise in plasma glucose concentration, the increase in plasma insulin is approximately threefold greater when glucose is administered orally compared with intravenously [1]. This alimentary enhancement of insulin release, known as the 'incretin' effect, is primarily humoral

and the peptides, glucagon-like peptide-1 (7-36) amide (GLP-1) and glucose-dependent insulinotropic peptide (GIP; formerly known as gastric inhibitory peptide), are the most important incretin hormones [1,2]. Both are potent insulinotropic hormones, released by oral glucose as well as by ingestion of mixed meals, and up to two-thirds of the insulin normally secreted in relation to a meal are thought to be as a result of the actions of these hormones.

GIP is a 42-amino acid peptide, processed from a precursor of 153 amino acids [3] and released from specific endocrine cells (K-cells), which, although their highest density is within the duodenum, are also found in the small intestine mucosa

Correspondence to: Professor S. R. Bloom, Department of Metabolic Medicine, Imperial College London, 6th Floor Commonwealth Building, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK.
E-mail: s.bloom@imperial.ac.uk

[4]. Oral ingestion of carbohydrates and lipids results in a 10–20-fold elevation of plasma GIP concentration. The interaction of GIP with its receptor, which is expressed on pancreatic B-cells, causes an elevation of cAMP leading to an increase in intracellular calcium-enhancing exocytosis of insulin-containing granules [5]. Although mice with a targeted deletion of the GIP receptor gene become glucose intolerant [6], immunoneutralization studies with GIP or studies employing a fragment GIP (7–30) amide, which acts as a GIP receptor antagonist, reduce the incretin effect by only 20–50% [7,8]. Therefore, GIP alone does not represent the full incretin effect. Furthermore, patients with resections of different parts of their small bowel show that the incretin effect does not correlate with secretion of GIP, supporting evidence that the distal bowel also releases an additional incretin [9].

The other incretin hormone, GLP-1, is normally synthesized in the intestinal L-cells of the distal ileal and colonic mucosa by tissue-specific post-translational processing of the glucagon precursor, preproglucagon [10], and is released into the circulation in response to a meal. The plasma concentration of GLP-1 rises threefold after a meal from a fasting level of approximately 15 pmol/l to a peak postprandial level of 40 pmol/l [1]. Injection into rats of the GLP-1 receptor agonist, exendin 9–39, results in a suppression of the incretin effect by 70% [11]. These studies indicate that GIP and GLP-1 are responsible for the full incretin effect. GLP-1 has also been shown to be essential for normal postprandial glucose homeostasis in humans [12] and its secretion throughout the day is highly correlated to the release of insulin [13]. In agreement with these observations, mice with a targeted deletion of the GLP-1 receptor become glucose intolerant and develop fasting hyperglycaemia [14]. GLP-1 is thought to be one of the most potent insulin-releasing substances known, exceeding that of GIP [15]. Its role is to stimulate meal-induced insulin secretion from the pancreas [16,17] and also enhance the first-phase insulin response [18]. The insulinotropic effect of GLP-1 is thought to be glucose dependent [19], and this dependence on blood glucose concentration at or above fasting glucose levels should mean that GLP-1 is incapable of causing profound hypoglycaemia. However, we have previously reported that exogenous GLP-1 administration can cause fasting hypoglycaemia [20] and that supraphysiological levels of GLP-1 in association with a GLP-1 secreting tumour can cause reactive hypoglycaemia [21], thereby causing, in both cases, the normal homeostatic mechanism maintaining fasting blood glucose to be overridden. Like GIP, GLP-1 acts via a G-protein-coupled receptor on the pancreatic B-cells which causes accumulation of cAMP, and most of its subsequent effects appear to be secondary to this [5].

There has been a great deal of interest in GLP-1 as a treatment for Type 2 diabetes as it possesses a number of potential advantages over existing agents (Table 1). In addition to stimulating insulin secretion, GLP-1 also promotes all steps in insulin biosynthesis, including gene transcription [22], thereby augmenting supplies of insulin for secretion. More recently, direct effects of GLP-1 on B-cell growth and survival have been

Table 1 Glucose-lowering actions of GLP-1

Process	Effects of GLP-1
Insulin secretion	Stimulation
Insulin biosynthesis	Up-regulated
Insulin gene expression	Enhanced
Pancreatic B-cell mass	Increased
Glucagon secretion	Inhibition
Gastric emptying	Delay
Appetite and food intake	Suppression

demonstrated in animal models, with GLP-1 stimulated proliferation [23,24] and differentiation of new B-cells [25,26] along with inhibition of B-cell apoptosis [27]. All these effects lead to increased B-cell mass. GLP-1 also suppresses glucagon secretion in a glucose-dependent manner and therefore is unlikely to impair the glucagon counter-regulatory response to hypoglycaemia [1,28]. GLP-1 may also enhance peripheral glucose disposal [29] and insulin sensitivity [30]. In addition, GLP-1 delays gastric emptying and secretion [31,32], thus reducing the postprandial glucose excursions by delaying nutrient delivery to the small intestine. Centrally administered GLP-1 inhibits food intake in rodents, an effect that is reversed by administration of the specific GLP-1 receptor antagonist, exendin 9–39 [33]. This raises the possibility that peripherally released GLP-1 may have access to the direct effects on the brain because circulating GLP-1 can access the GLP-1 receptors in the brain areas (subfornical organ, area postrema) that participate in the regulation of appetite and energy homeostasis [34]. In humans, GLP-1 infusions dose dependently reduce food intake and have a satiating effect, although the effects of GLP-1 on the rate of gastric emptying may contribute to this [35]. In combination, these actions give GLP-1 a highly desirable profile as a glucose-lowering agent, particularly because of its effect on B-cell growth and restoration, the low risk of hypoglycaemia, reduction in appetite and suppression of glucagon, which is usually high in patients with diabetes and contributes to the hyperglycaemia.

The incretin effect in Type 2 diabetes

It is now recognized that inadequate secretion of insulin is a very early element in the development of Type 2 diabetes and the progression is because of declining B-cell function [36–38]. The B-cell defect is partly as a result of loss of B-cells, which may account for 50% of the reduction in insulin secretion in advanced Type 2 diabetes [39], but also because of impaired insulin secretion. Patients with Type 2 diabetes exhibit an almost complete loss of the incretin effect [40] and it is hypothesized that deficient incretin secretion in Type 2 diabetes may contribute to the pathogenesis of the disease.

As GIP and GLP-1 together are responsible for the incretin effect in healthy subjects, the incretin defect in Type 2 diabetes

could theoretically be because of impaired secretion, accelerated metabolism or defective function of the incretin hormones. There are a number of publications which report increased, decreased or normal secretion of GIP in Type 2 diabetes [41]. The GLP-1 secretion patterns in Type 2 diabetes are also controversial. They have been reported to be increased [42,43], decreased [44] and unaltered [15,45] in these patients. However, the early GLP-1 assays were unable to distinguish between GLP-1 secreted from the gut and GLP-1 immunoreactive molecules secreted from the pancreas as inactive by-products of glucagon secretion [such as GLP-1 (1-36) amide or (1-37) amide], the major proglucagon fragment or NH₂-terminally deleted GLP-1 (9-36) amide [46]. However, in one study a small group of identical twins, discordant for Type 2 diabetes, reported that the GLP-1 response was lower in the diabetic twin [47]. Based on the decreased GLP-1 secretion, it could be hypothesized that GLP-1 could be used as a treatment for Type 2 diabetes and the therapeutic potential of GLP-1 has been proposed following a number of studies. Repeated intravenous infusion (1–1.2 pmol/kg/min) can normalize fasting blood glucose in patients with Type 2 diabetes, even in individuals with poor B-cell reserve [48] and Larsen *et al.* infused GLP-1 (4 ng/kg/min to 1.2 pmol/kg/min) continuously for 7 days to a group of Type 2 diabetic patients leading to improvements in blood glucose measurements [49]. We have also previously demonstrated a significant improvement in postprandial glycaemic control when GLP-1 is given subcutaneously over a 3-week period [50]. In addition, Zander *et al.* have also shown that continuous subcutaneous GLP-1 administration (4.8 pmol/kg/min), over 6 weeks, is a highly effective treatment for Type 2 diabetes, causing marked improvements in the glycaemic profile with a reduction in HbA_{1c} of 1.2% and weight loss. The beneficial effects of continuous subcutaneous administration of GLP-1 over 3 months have also been confirmed in elderly patients with Type 2 diabetes [51]. In addition, insulin sensitivity almost doubled and insulin secretion increased, indicating an improvement in B-cell function [52].

GLP-1 metabolism

GLP-1 is metabolized extremely rapidly in the circulation, with a half-life *in vivo* of less than 2 min [53], and is the probable explanation for the short-lived effect of single doses of native GLP-1. The ubiquitous enzyme, dipeptidyl peptidase IV (DPP-IV), a membrane-bound ectoenzyme found at several sites, including the kidneys, intestine and capillary endothelium, causes NH₂-terminal degradation of the peptide and cleaves GLP-1 at the penultimate alanine residue to generate GLP-1 (9-36) amide [46,54] (Fig. 1). As this residue is important for receptor activation, DPP-IV may be involved in regulating the biological activity of GLP-1 [54] and, in addition, pharmacological studies with the metabolite, GLP-1 (9-36) amide, suggest that it behaves as a functional antagonist at the pancreatic receptor [55]. However, further *in vivo* studies have not confirmed antagonism of the insulinotropic effect of GLP-1 in humans [56].

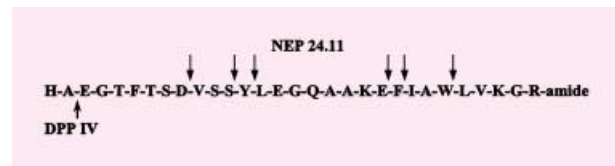


FIGURE 1 The amino acid sequence of GLP-1 (7-36), showing the cleavage sites of DPP-IV and NEP 24.11 [97]. Copyright © 2004 American Diabetes Association. From *Diabetes* 2004; 53: 2181–2189. Reprinted with permission from The American Diabetes Association.

GIP is also metabolized by DPP-IV, which cleaves the dipeptide at the NH₂-terminal of the GIP molecule, and which is therefore inactivated [53]. The metabolite, GIP 3-42 amide, may also act as an antagonist at its own receptor [57]. However, the two hormones differ in their sensitivities to DPP-IV. Whereas as little as 10% of exogenous GLP-1 survives in intact form, as much as 50% of the infused GIP survives [58,59] and the half-life of GIP is 7 min compared with a GLP-1 half-life of less than 2 min [59,60].

Given the interest in developing therapeutic strategies based on GLP-1, this has prompted studies based on examining GLP-1 metabolism. Mice lacking the DPP-IV enzyme (DPP-IV knockout) show better glucose tolerance, higher GLP-1 levels and greater insulin sensitivity than their wild-type equivalents [53,61]. Both subcutaneously and intravenously administered GLP-1 are rapidly degraded to GLP-1 (9-36) amide; the latter accounting for 80% of the increase in immunoreactivity following exogenous administration [58]. Furthermore, DPP-IV is localized in the endothelium of capillaries adjacent to the intestinal L-cells that produce GLP-1, and up to 50% of the synthesized GLP-1 is NH₂-terminally degraded as it enters the DPP-IV-containing blood vessels draining the intestinal mucosa [62].

GLP-1 is also a substrate for a second enzyme, neutral endopeptidase (NEP) 24.11 *in vitro* [63,64], which is a membrane-bound zinc metallopeptidase that also cleaves the peptide at the NH₂-terminal aromatic ring or hydrophobic amino acids. Six potential cleavage sites have been identified [63] (Fig. 1). This enzyme is found in high concentrations in the kidneys and may be involved in the renal clearance of the peptide hormone.

Therapeutic strategies based on GLP-1

As discussed above, because GLP-1 is metabolized rapidly to the inactive truncated form GLP-1 (9-39) by DPP-IV, the native peptide is an unlikely glucose-lowering agent and cannot be used clinically. Therefore, therapeutic strategies have focused on the development of DPP-IV-resistant analogues (or agonist at the GLP-1 receptor) and selective DPP IV inhibitors. Intravenous infusion of GLP-1 efficiently lowers plasma glucose in patients with Type 2 diabetes, but needs to be given continuously and therefore is impractical. Continuous subcutaneous infusions are currently the only way to obtain stable high plasma concentrations of GLP-1 in long-term studies.

Therefore, there are two therapeutic strategies to overcome this obstacle in designing GLP-1 treatments for Type 2 diabetes: (i) GLP-1 receptor agonistic peptides or 'GLP-1 mimetics' which are resistant to DPP-IV, therefore avoiding rapid enzymatic degradation and prolonging circulation time; and (ii) selective DPP-IV enzyme inhibition to prevent GLP-1 degradation and improve circulation time.

Enzyme-resistant GLP-1 analogues

These analogues are resistant to DPP-IV degradation and therefore prolong circulation time and include exendin-4 and albumin-bound GLP-1 derivatives. This approach has been investigated experimentally, and promising compounds now have Food and Drug Administration (FDA) approval whilst others are in the final stages of clinical development.

Exendin-4

Exendin-4 was originally isolated from the venom of a gila monster, *Heloderma* lizard species, and has been the most extensively investigated. Exendin-4, which shares 53% amino acid homology with native GLP-1, is a more potent agonist at the mammalian GLP-1 receptor, because of its resistance to DPP-IV degradation. In contrast to GLP-1, exendin-4 has a glycine residue at its penultimate position at the NH₂-terminal instead of alanine as in GLP-1 and therefore the plasma half-life is 26 min in humans [65] compared with 1–2 min for intact biological GLP-1 [66]. However, exendin-4 is also a poor substrate for NEP 24.11, because, although the NH₂-terminal regions of both peptides show high sequence homology, several potential NEP 24.11 cleavage sites present in GLP-1 are absent on exendin-4 [63] and this may also contribute to the prolonged half-life of exendin-4. In addition, exendin-4 is larger than GLP-1 by virtue of its COOH extension (39 amino acids), which may also contribute to a being a poorer substrate for NEP 24.11. The metabolic clearance of exendin-4 is similar to the glomerular filtration rate, suggesting that the kidneys are important for clearing exendin-4. In animal models of diabetes, exendin-4 causes a reduction in plasma glucose, HbA_{1c} and body weight gain [67,68]. Exendin-4 also increases pancreatic B-cell mass both in rats with a genetic deficiency in neonatal B-cells [69] and in pre-diabetic *db/db* mice via increased proliferation and decreased apoptosis [70]. In healthy volunteers, acute infusions of exendin-4 are insulinotropic, reduce both fasting and postprandial glucose concentrations, and delay gastric emptying [65]. Exendin-4 also reduces the caloric intake in the absence of symptoms of nausea and therefore these results suggest that exendin-4 may be a useful treatment for Type 2 diabetic patients, particularly for obese individuals [65].

Exenatide (previously known as the investigational agent AC2993, a synthetic exendin-4) has been assessed as treatment for Type 2 diabetes in phase 3 clinical trials (the AMIGO studies) [74–76] sponsored by Amylin Pharmaceuticals Inc. (San Diego, CA, USA) and was approved for treatment of

Type 2 diabetes by the FDA in April 2005. In a placebo-controlled study in Type 2 diabetic patients, exenatide reduces fasting plasma glucose when given acutely, and postprandial glucose when given twice daily at breakfast and dinner over 5 days [71]. However, during the 5-day study, there was no significant effect on fasting blood glucose, suggesting that the duration of action of the evening dose was inadequate to maintain the reductions in blood glucose overnight. This has been confirmed in a 1-month study in which 10 patients with Type 2 diabetes (aged 44–72 years, mean fasting glucose 11.4 ± 0.9 mmol/l) were given daily or twice-daily subcutaneous exenatide. Once-daily injections did not achieve satisfactory glucose control, but twice-daily injections significantly improved HbA_{1c} (reduction from 9.1 to 8.6%) despite lack of full 24-h blood glucose control [72]. The clinical usefulness of exendin-4 was assessed in a phase 2 clinical trial in which 109 patients with Type 2 diabetes, already taking sulphonylurea and/or metformin, were prescribed exenatide (0.08 µg/kg) injected subcutaneously two or three times per day for 4 weeks [73]. In all treated groups, there was a reduction in HbA_{1c} of 0.7–1.1%. The most common side-effect was transient mild to moderate nausea in 30% of patients. Although mild hypoglycaemia was reported in about one-third of patients also taking sulphonylureas, this was not confirmed on blood-glucose monitoring. Recently, phase 3 studies (the AMIGO studies) have determined the effects of subcutaneous exenatide at a dose of 5 or 10 µg twice daily given for 30 weeks in addition to sulphonylurea therapy [74], metformin therapy [75] or both [76] in patients with Type 2 diabetes. The initial mean HbA_{1c} in the three studies was 8.2–8.6%. A reduction in HbA_{1c} of 0.8–0.9 and 0.4–0.6% in the 10 and 5 µg arms, respectively, compared with increases of approximately 0.1% in the placebo groups. There was also a dose-dependent progressive weight loss, with an end-of-study loss in the 10 µg exenatide arms of –1.6 to –2.8 kg and –0.9 to –1.6 kg in the 5 µg exenatide arms. The results of the exenatide plus metformin study are shown in Fig. 2. Exenatide was generally well tolerated with the most frequent adverse event being mild to moderate nausea affecting between 30 and 50% and 30–40% of the patients in the 10- and 5-µg exenatide arms, respectively. However, the nausea was usually transient and resolved after the first few weeks, with less than 10% experiencing nausea in the last few weeks of the trials. The incidence of severe nausea was uncommon (3–5% of cases) with a low incidence of withdrawals because of nausea (4% in the 10-µg arm, 2% in the 5-µg arm and < 1% in the placebo arms) in all three studies. Mild to moderate hypoglycaemia was more common in the exenatide-treated groups (approximately 30% in the 10-µg groups and 14–20% in the 5-µg groups), but severe hypoglycaemia was rare and there was only one withdrawal because of severe hypoglycaemia.

In addition to these studies, a recent report has compared the effects of exenatide (10 µg twice daily) with insulin glargine (once daily, titrated until fasting blood glucose levels were < 5.6 mmol/l) for 26 weeks in 551 patients with sub-optimally controlled Type 2 diabetes (initial mean HbA_{1c} 8.2 and

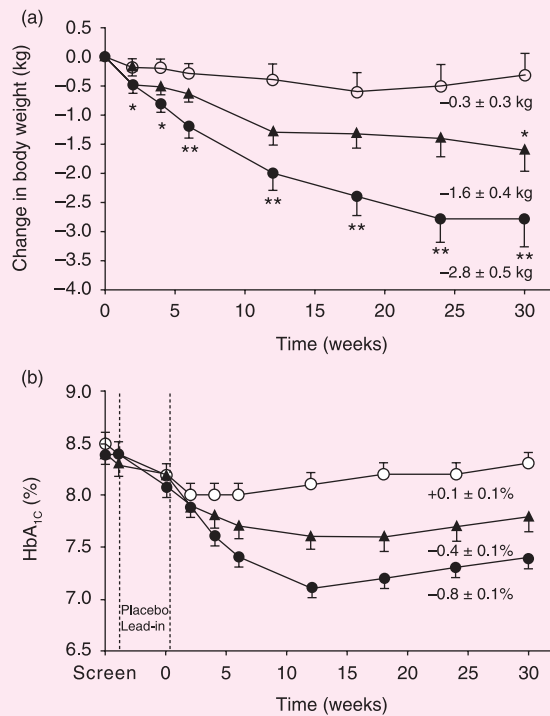


FIGURE 2 Change in body weight from baseline (a) and HbA_{1c} values over the course of the study (b) in the placebo (○), 5 µg exenatide (▲) and 10 µg exenatide (●). Data are mean ± SEM [75]. * $P \leq 0.05$; ** $P \leq 0.001$ compared with placebo treatment. Copyright © 2005 American Diabetes Association. From *Diabetes Care* 2005; 28: 1092–1100. Reprinted with permission from The American Diabetes Association.

8.3% in the exenatide- and insulin-treated groups, respectively) [77]. At week 26, HbA_{1c} had fallen by 1.11% in both the glargine and exenatide groups. Exenatide reduced postprandial glucose excursions more than insulin glargine, but glargine reduced fasting blood glucose concentrations more than exenatide. However, there was a –2.3-kg weight loss with exenatide compared with a 1.8-kg weight gain with glargine. The rates of hypoglycaemic episodes were similar in the two groups. However, nocturnal hypoglycaemia was more frequent in the insulin-treated group, but exenatide had a higher rate of gastrointestinal adverse effects (particularly nausea) occurring in 30% of patients, with severe nausea effecting 5%. Again this adverse effect was transient, with only 8% experiencing nausea in the last 8 weeks of the study. It should be noted that twice-daily injections of exenatide do not provide full 24-h exposure to the GLP-1 receptors and may explain the less conspicuous effect on fasting plasma glucose with exenatide compared with a continuous infusion of native GLP-1. However, a more pronounced and gradual fall in HbA_{1c} is observed, and this may indicate that the mean 24-h plasma glucose levels are lowered relatively more than the fasting plasma glucose. In view of this, Amylin Pharmaceuticals Inc. has announced that a slow-release formulation is in develop-

ment. Also, exenatide has not yet been assessed as monotherapy, but only as an adjunct to existing therapies, where native GLP-1 has been shown to exert additive or even synergistic effects.

Albumin-bound GLP-1 derivatives

Another approach in designing GLP-1 treatments for Type 2 diabetes has been to bind a GLP-1 analogue to albumin to slow absorption and elimination. Novo Nordisk have developed an acetylated derivative of the GLP-1 which binds non-covalently to albumin and has a half-life of longer than 10 h, enabling it to be given once a day. The compound is liraglutide (formerly NN2211) and consists of native GLP-1 in which the C16 acyl chain is attached via a glutamoyl spacer to lysine residue 26 (while lys34 is substituted by Arg) : Arg(34)lys(26)-[N-epsilon-[gamma-Glu(N-alpha-hexacanoyl)]]-GLP-1(7-37). The compound shows slow release from the subcutaneous injection site and binds to albumin, which renders it resistant to DPP-IV and allows the bound fraction at least to escape renal elimination. This has resulted in a half-life of 10–12 h in both control and Type 2 diabetic subjects and, thereby, adequate 24-h exposure after a single injection [78,79]. In a study by Juhl *et al.*, 11 patients with recently diagnosed Type 2 diabetes (mean age 59 years, HbA_{1c} 6.5% and mean duration of diabetes 2.7 years), received liraglutide as a single subcutaneous dose (10 µg/kg) into the abdomen at 23.00 h [79]. Blood samples were taken in both the fasting and postprandial state to assess the effect of liraglutide on plasma glucose, glucagon and insulin secretion rates (ISR). Gastric emptying was assessed by co-administration of 3-ortho-methylglucose (3-OMG) with a 2500-kJ meal on the next day at 11.00 h. Liraglutide not only effectively reduced both fasting plasma glucose by 1.2 mmol/l (to a mean of 6.9 mmol), but also decreased plasma glucose excursion after a standard mixed meal by modifying insulin secretion, delaying gastric emptying and suppressing glucagon secretion. The half-life of liraglutide was confirmed at 10 ± 3.5 h. Two patients, with the highest peaks of liraglutide, experienced treatment-associated nausea, one mild and the other moderate. In another study, a single dose of liraglutide (7.5 µg/kg) given to patients with Type 2 diabetes was shown to restore pancreatic B-cell responsiveness to physiological hyperglycaemia [80]. Results from longer-duration studies have also reported that, in patients with Type 2 diabetes, treatment with once-daily liraglutide (6 µg/kg) for 1 week significantly reduces 24 h glucose concentrations, glucagon levels and improves B-cell function when compared with placebo [81]. In contrast to earlier studies, gastric emptying was not delayed and may reflect the lower dose used in this study. The beneficial effects appear to be maintained over longer periods of treatment. In two studies in which 190 and 210 patients with Type 2 diabetes were randomly assigned to five doses of liraglutide (0.045, 0.225, 0.45, 0.6 or 0.75 mg) as a single dose, placebo and either sulphonylurea treatment (glimepiride 1–4 mg) [82] or metformin 1 g twice daily for 12 weeks [83]. In the liraglutide and glimepiride study there was a reduction in HbA_{1c} in all but the

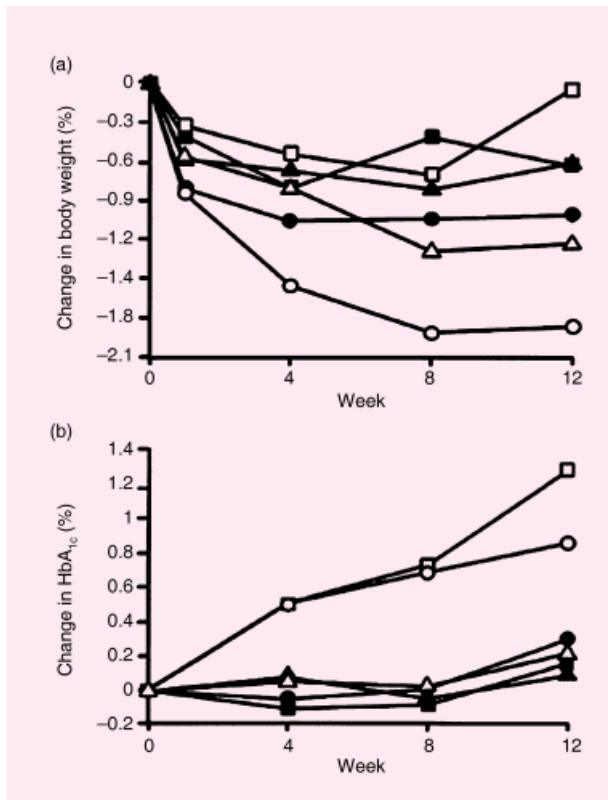


FIGURE 3 Percentage change in body weight (a) and HbA_{1c} (b) over time. Liraglutide 0.045 mg (□), 0.225 mg (○), 0.45 mg (△), 0.6 mg (■), 0.75 mg (●), metformin (▲) [83]. From *Diabet Med* 2005; 22:1016–1023. Reprinted with permission from Blackwell Publishing.

lowest liraglutide dosage group, with a reduction of 0.75% in the liraglutide 0.75-mg group. There was also a reduction in fasting plasma glucose of 1.8 mmol/l compared with placebo, and mean B-cell function was significantly improved in the 0.75-mg liraglutide group, although insulin resistance did not change [82]. In addition, there was a decrease in body weight by 1.2 kg in the 0.45-mg liraglutide group compared with placebo. In patients treated with glimepiride, fasting plasma glucose and HbA_{1c} decreased but body weight increased slightly [82]. In the liraglutide and metformin study [83], baseline glycaemic control was good except at the two lowest doses of liraglutide (0.045 mg and 0.225 mg) (Fig. 3) [83]. After 12 weeks, in the liraglutide groups, a weight change of -0.5 to -1.9% was noted compared with -0.61% in the metformin group (Fig. 3). The weight loss observed in the liraglutide group was because of loss of fat cell mass rather than lean body mass [83]. Liraglutide was well tolerated with no major hypoglycaemic episodes in either study. The risk of nausea and vomiting was rare (< 10%) and did not cause any discontinuations of the clinical trial therapy in either study [82,83].

Another study in which liraglutide (0.6 mg) was given once daily for 8 weeks to type 2 diabetic patients (mean age 61 years, HbA_{1c} 7.6%) also demonstrated a reduction in fasting serum glucose and HbA_{1c} [-0.33%]. In addition, although there was

a non significant reduction in body weight, results from DEXA scanning indicated that fat mass decreased and lean tissue mass increased after treatment with liraglutide compared with placebo [84]. Pancreatic B-cell function was improved, but insulin resistance, 24 h energy expenditure and gastric emptying were unchanged. The injections were well tolerated with no hypoglycaemic episodes and the most common side-effect was nausea and vomiting (usually transient) [84].

From the available data, protease-resistant GLP-1 analogues appear to have remarkably few undesirable side-effects. Nausea and vomiting are the commonest reported side-effects, which is not surprising given that these compounds are based on naturally occurring GLP-1 which is known to delay gastric emptying. However, it is noteworthy that even this symptom is generally reported as being transient, occurring primarily in the first week and then disappearing, suggesting tachyphylaxis to the gastrointestinal effects occurs. Importantly, no serious hypoglycaemic events have been reported and liraglutide does not impair the glucagon-mediated counter-regulatory response to hypoglycaemia.

DPP-IV enzyme inhibitors

The alternative approach is the use of selective DPP-IV enzyme inhibitors to prevent GLP-1 degradation and improve circulation time, and these have been the focus of much interest. The finding that GLP-1 is uniquely sensitive to DPP-IV cleavage *in vivo* has led to a number of pharmaceutical companies developing selective compounds to inhibit DPP-IV activity and, thereby, enhancing the biological activity of incretin hormones. Multiple DPP-IV inhibitors have been characterized and several potent orally active compounds have already been described. These compounds lower blood glucose in diabetic rodent models via prolongation of GLP-1 and GIP action [85–88]. In humans, in the first chronic study, the short-acting Novartis DPP-IV inhibitor (Novartis Pharmaceuticals UK Ltd, Frimley, UK), NVP DPP728, was given two or three times per day for 4 weeks to patients with recently diagnosed Type 2 diabetes. Both fasting and postprandial glucose concentrations were significantly reduced and HbA_{1c} was also lowered after only 4 weeks of treatment [89]. NVP DPP728 was well tolerated with very few side-effects, which were described as mild and transient and did not necessitate discontinuation of treatment. Novartis has subsequently introduced another inhibitor, LAF237 (Vildagliptin), which has a longer duration of action and is therefore suitable for once-daily administration. It has now reached phase 3 clinical development in patients with Type 2 diabetes [90]. This compound, given as a single 100-mg dose daily for 4 weeks, caused significant improvements in both fasting and postprandial glucose concentrations, and lowered HbA_{1c} to a similar extent as the NVP DPP728 administered two or three times per day [90]. In addition, glucagon levels were significantly lowered by LAF237, suggesting that GLP-1-mediated lowering of glucagon, in addition to the insulinotropic effect, contributes to the glucose-lowering effect

of the DPP-IV inhibition. This compound was well tolerated and there were no significant side-effects, as with NVP DPP728. More recently, Ahren *et al.* have reported that LAF237 (50 mg), given once daily to patients with Type 2 diabetes treated with metformin (mean HbA_{1c} 7.7 ± 0.1%), reduced HbA_{1c} by 0.6 ± 0.1%, with reductions in mean fasting and postprandial glucose levels of 1.2 ± 0.4 and 2.2 ± 0.4 mmol/l, respectively, when the drug was administered for 12 weeks [91]. At 52 weeks, mean change in postprandial and fasting blood glucose was -2.4 ± 0.6 and -1.1 ± 0.6 mmol/l, respectively. HbA_{1c} did not change after week 12 in the LAF237-treated patients, but increased in the placebo group, and the between-group difference at 1 year was -1.1 ± 0.2% [91]. However, there was no effect on body weight at the end of 12 weeks or 1 year in the LAF237-treated group. At 12 weeks, body weight decreased by 0.4 ± 0.2 and 0.5 ± 0.2 kg in the LAF237 plus metformin group and the metformin group alone, respectively, and by 0.2 kg in both groups at the end of 1 year. A further study has demonstrated that LAF237 (50 mg) given daily, in addition to metformin, produced an improvement in pancreatic B-cell function and insulin sensitivity that was sustained throughout the 52-week study period. This suggests that these latter mechanisms may contribute to the improved glycaemic control following LAF237 treatment [92]. In both these studies, LAF237 was well tolerated and the overall incidence of adverse side-effects was similar in the two groups of patients. In particular, LAF237 does not appear to be associated with hypoglycaemic episodes.

These results are very encouraging. However, there are many substrates for DPP-IV [93] and therefore the possibility of side-effects unrelated to incretin hormone metabolism, giving rise to undesirable adverse effects, has also been of some concern. In addition to stabilizing the incretins, GLP-1 and GIP, DPP-IV inhibitors also prolong the action of a number of neuropeptides, including pituitary adenylylate cyclase-activating polypeptide (PACAP), neuropeptide Y (NPY), substance P, growth hormone-releasing hormone and chemokines such as stromal cell-derived factor 1 and macrophage-derived chemokine. Potential side-effects resulting from prolongation of the action of these messengers include neurogenic inflammation (substance P, NPY), increase in blood pressure (NPY), enhanced inflammation and allergic reactions (chemokines). DPP-IV has also been found as a membrane-associated molecule on the surface of T-cells (where it is known as CD26) and contributes to T-cell activation and proliferation via its action with other membrane-expressed antigens such as CD45 [94], raising the possibility that DPP-IV inhibitors may compromise immune function. Therefore, given the diverse effects, particularly on the immune and endocrine systems, the long-term safety of DPP-IV inhibitors merits careful consideration and analysis.

There are no studies to date with DPP-IV inhibitors as monotherapy, but it is likely that these agents would be particularly useful in patients with recently diagnosed Type 2 diabetes and for diabetes prevention, and that DPP-IV inhibitors would not be very useful in long-duration Type 2 diabetes. This could be because of impaired GLP-1 secretion in long-standing Type 2

diabetes [95], along with both poor insulin secretory capacity and sensitivity and insensitivity to GLP-1 in these patients [96].

In summary, GLP-1-based therapy possesses a number of potential advantages over existing agents for the treatment of Type 2 diabetes, particularly in terms of the effects on pancreatic B-cell growth, potential weight loss and hypoglycaemic risk. The available data shows that DPP-IV-resistant GLP-1 analogues and DPP-IV inhibitors are remarkably efficacious and are well tolerated, with transient nausea and vomiting being the commonest reported side-effect. However, as there is the risk of potential adverse effects of DPP-IV inhibitors, particularly on the immune and endocrine systems, the long-term safety of DPP-IV inhibitors warrants careful consideration and analysis. In addition, studies demonstrating whether these agents will also be useful as monotherapy are awaited.

Competing interests

None declared.

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