Liraglutide: a human GLP-1 analog for Type 2 diabetes

Glucagon-like peptide-1 (GLP-1) is an incretin hormone secreted by the intestinal mucosa in response to nutrient ingestion. Although GLP-1 has multiple potentially beneficial effects, it is unsuitable as a therapy in Type 2 diabetes due to its short half-life. Liraglutide is an analog of human GLP-1 with high (97%) sequence homology, but has protracted pharmacokinetics as a result of aggregation at its injection site, albumin binding in the circulation and reduced susceptibility to enzymatic degradation. Liraglutide has glucose-lowering effects spanning 24 h, with improvements in fasting and postprandial glucose levels. In clinical studies, liraglutide has achieved reductions in HbA_{1c} of up to 1.6% when used as monotherapy in drug-naive subjects. The incidence of hypoglycemia with liraglutide treatment is relatively low, perhaps reflecting a glucose dependency of its insulinotropic action. Liraglutide also has clinically relevant effects on body weight, with mean reductions of 2–3 kg occurring in large clinical trials. Additionally, data are suggesting beneficial effects on cardiovascular risk factors: systolic blood pressure is reduced by 2–6 mmHg with liraglutide treatment, while Phase II studies have demonstrated improvements in molecular markers of cardiovascular risk. Treatment with liraglutide also appears to have effects on β-cell function, with clinical data showing improvements in β-cell function and insulin secretory capacity. The significance of animal data suggesting beneficial effects of liraglutide on β-cell mass and apoptosis have not been confirmed in humans. The most common adverse event during liraglutide treatment is nausea; this is experienced initially by a minority of subjects, but decreases to relatively low levels with continued treatment.

KEYWORDS: cardiovascular ▪ exenatide ▪ GLP-1 ▪ glucose lowering ▪ LEAD trials ▪ liraglutide ▪ Type 2 diabetes ▪ weight loss

Tina Vilsbøll
Department of Internal Medicine F, Gentofte Hospital, Niels Andersens Vej 65, Hellerup, Copenhagen DK-2900, Denmark
Tel.: +45 4094 0825
Fax: +45 3977 7661
t.vilsboll@dadlnet.dk

The need for new therapy in Type 2 diabetes

Type 2 diabetes is a complex multisystem disorder, defined by insulin resistance and failure of pancreatic β cells to produce sufficient insulin in order to maintain blood glucose levels within acceptable limits. Furthermore, fasting and postprandial hyperglucagonemia in patients with Type 2 diabetes have been shown to result in increased glucagon-induced hepatic glucose production, which again contributes to fasting hyperglycemia and exaggerated postprandial glucose excursions. The deficiencies in Type 2 diabetes rarely occur alone: patients with Type 2 diabetes are often obese and frequently have hypertension and dyslipidemia. This constellation of risk factors, together with the damaging effects of hyperglycemia, places many patients with Type 2 diabetes at cardiovascular risk. Comparisons with age-matched, normoglycemic populations suggest that the presence of elevated blood glucose concentration amplifies the risk posed by other cardiovascular risk factors for serious outcomes such as coronary events and stroke [101]. Type 2 diabetes is also a progressive disease, characterized by a decline in β-cell function that occurs over a period of years. As a result, all current therapies for Type 2 diabetes need to be adjusted, upgraded or substituted as the disease progresses, in order to maintain glycemic control. The current treatment paradigm in Type 2 diabetes requires initial treatment with dietary and lifestyle modifications, usually rapidly supplemented by oral antidiabetic agent therapy. The biguanide metformin is a common first step in pharmacotherapy, alternatives being insulin secretagogues. The progressive nature of the disease means that any single oral antidiabetic agent is frequently supplemented by a second, and subsequently perhaps a third, agent; many patients with Type 2 diabetes ultimately progress to therapy with insulin as a necessary step in maintaining glycemic levels within acceptable limits.

These current therapies for Type 2 diabetes are not without limitations. Many agents – sulfonylureas, thiazolidinediones and insulin – have a tendency to cause weight gain, hardly desirable in a patient population in which many are already

future medicine part of fsg

obese. The agents with the most pronounced efficacy in glucose lowering also have a tendency to cause hypoglycemia. Several agents have important contraindications that restrict their use in renally impaired or other special patient populations, while none has proven capable of indefinitely preventing the progression of Type 2 diabetes.

Physiological & therapeutic effects of glucagon-like peptide-1

Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are peptide hormones secreted from the small intestine and colon in response to oral, but not intravenous, glucose administration. The increased secretion of insulin resulting from the effects of these hormones on the pancreatic β cells – known as the incretin effect – accounts for up to 70% of the postprandial insulin response in healthy individuals, and is accompanied by suppression of glucagon secretion [1,2].

GLP-1 has a number of acute effects on glucose sensing and insulin secretion by the β-cell, including closure of ATP-dependent potassium channels, shifting membrane potential and sensitizing β cells to glucose [2], reduction of activity of the voltage-dependent potassium channel [3], release of Ca^{2+} from internal stores [4] and an increase in the number of readily releasable insulin secretory vesicles. Chronic effects of GLP-1 include increased insulin mRNA levels [5], stimulation of insulin transcription [6] and stabilization of insulin mRNA, and increased levels of transcription factor PDX-1 mRNA and protein [7,8]. GLP-1 thus provides both an immediate effect on insulin secretion and longer-term stimulation of insulin synthesis. Longer-term effects of GLP-1 seem to be at the level of increased β-cell neogenesis (through differentiation of duct cells [7]), proliferation of existing β cells [9], and through reduction in lipotoxicity- and cytokine-mediated β-cell apoptosis [10]. GLP-1 also delays gastric emptying and increases satiety, resulting in weight loss [11,12].

The physiological role of GLP-1, together with a deficiency of GLP-1 secretion seen in Type 2 diabetes [13], suggests it as a interesting potential therapy for that disease, and indeed infusion of GLP-1 can lower levels of blood glucose [14]. However, native GLP-1 has a short half-life of less than 2 min following administration *in vivo*, being rapidly degraded by the enzyme dipeptidyl peptidase-4 (DPP-4) [15,16]. Therapeutic administration of GLP-1 is thus impractical, and efforts have focused on amending the pharmacokinetic properties of GLP-1 in a series of derivatives and analogs.

Design & pharmacology of liraglutide

Native GLP-1 is a 30-amino acid peptide produced by cleavage of the transcription product of the preproglucagon gene [17]. Liraglutide is a recombinant, acylated analog of human GLP-1. The native structure of the GLP-1 molecule has been modified by replacement of lysine with arginine at position 34 and attachment of a C16 acyl chain at position 26 via a glutamoyl spacer. Liraglutide thus has 97% sequence homology to native GLP-1. Structure–activity data supporting the specific modifications in the liraglutide molecule were reported by Knudsen and colleagues, who varied the position of attachment of the γ-glutamic acid spacer and C16 fatty acid, achieving half-lives of up to 20 h [18]. Liraglutide molecules form a complex tertiary structure in concentrated solution, with seven molecules forming regular heptamer aggregates that may be compared with the hexamers found in concentrated solutions of insulins. The heptamers of liraglutide are believed to form micellar aggregates at the subcutaneous injection site. Protraction of liraglutide concentration appears to be due to a combination of albumin binding in the circulation, aggregation at the injection site and reduced susceptibility to DPP-4 degradation [19,20]. Together, these aggregative properties lead to prolonged release of liraglutide monomers into the circulation. Once there, the acyl side-chain allows reversible binding to albumin molecules. As a result, liraglutide in the circulation is less susceptible to degradation by DPP-4 [19], and the pharmacokinetics of liraglutide render it suitable for once-daily administration, with a half-life of 13 h. Pharmacokinetics of liraglutide have been found to be independent of age and gender [21].

Glycemic control & hypoglycemia

Pharmacodynamic studies have shown that liraglutide has a glucose-lowering effect spanning 24 h. In the 1-week crossover study by Degn and colleagues, liraglutide achieved reductions in glucose levels during fasting, prandial and nocturnal periods, with significant improvements in fasting glucose levels, postprandial glucose excursions and 24-h total area under the curve (AUC) for glucose [22]. Stimulation of insulin secretion with liraglutide also shows a glucose dependency. Nauck *et al.* conducted a series of stepwise hypoglycemic clamps in 11 patients with Type 2 diabetes following injection of liraglutide or placebo [23]. They found that insulin secretion was increased by liraglutide relative to placebo at higher glucose levels (4.3 and 3.7 mmol/l), but not at lower

glucose levels (3.0 or 2.3 mmol/l). The glucagon response to hypoglycemia was unaffected by liraglutide treatment.

A 5-week study in 144 patients with Type 2 diabetes by Nauck and colleagues found that liraglutide treatment in combination with metformin produced a 3.9 mmol/l reduction in fasting glucose levels, significantly greater than with metformin alone or metformin plus glimepiride [24]. Similar findings resulted from a 12-week study by Madsbad and colleagues, in which fasting glucose levels were reduced by 1.8 mmol/l ($p < 0.001$ vs placebo) and HbA_{1c} levels reduced by 0.75% ($p < 0.0001$) [25]. No major hypoglycemic episodes occurred with liraglutide in either study.

Liraglutide as monotherapy

Effects of liraglutide on glycemic control and hypoglycemia were studied in 163 patients with Type 2 diabetes in a 14-week Phase II study [13]. In this double-blind, randomized study, three dose levels of liraglutide (0.65, 1.25 and 1.9 mg, all once daily) were compared with placebo. Subjects included were receiving one oral antidiabetic agent prior to the study or were treated with diet alone. A dose-dependent reduction in HbA_{1c} was evident with liraglutide treatment, the highest dose reducing mean HbA_{1c} by 1.7% relative to placebo (baseline 8.5%).

The clinical effects of liraglutide treatment have been further investigated in the Liraglutide Effect and Action in Diabetes (LEAD) series of Phase III studies including more than 4000 patients with Type 2 diabetes (Figure 1).

Monotherapy with liraglutide was evaluated in the LEAD 3 study, a double-blind, randomized, controlled trial in 746 patients during 52 weeks of treatment. Patients included were those inadequately controlled on diet and exercise, or with no more than half-maximal dose of one oral antidiabetic agent (which was discontinued at entry) [26]. The two liraglutide dose levels, 1.8 and 1.2 mg once daily, achieved reductions in HbA_{1c} of 1.1 and 0.8%, respectively, when looking at the total population; each of these reductions being significantly greater than the 0.5% reduction achieved with glimepiride, 8 mg daily ($p < 0.0001$ and $p = 0.0014$; Figure 2). Patients who were on diet and exercise at entry had sustained HbA_{1c} reductions with liraglutide of up to 1.6%. Postprandial glucose levels were reduced by 2.1 mmol/l and fasting glucose by 1.4 mmol/l from baseline on a dose of 1.8 mg.

Combination therapy

Improved glycemic control has also been reported when liraglutide is used in combination with oral antidiabetic therapy. In LEAD 2, a double-blind,

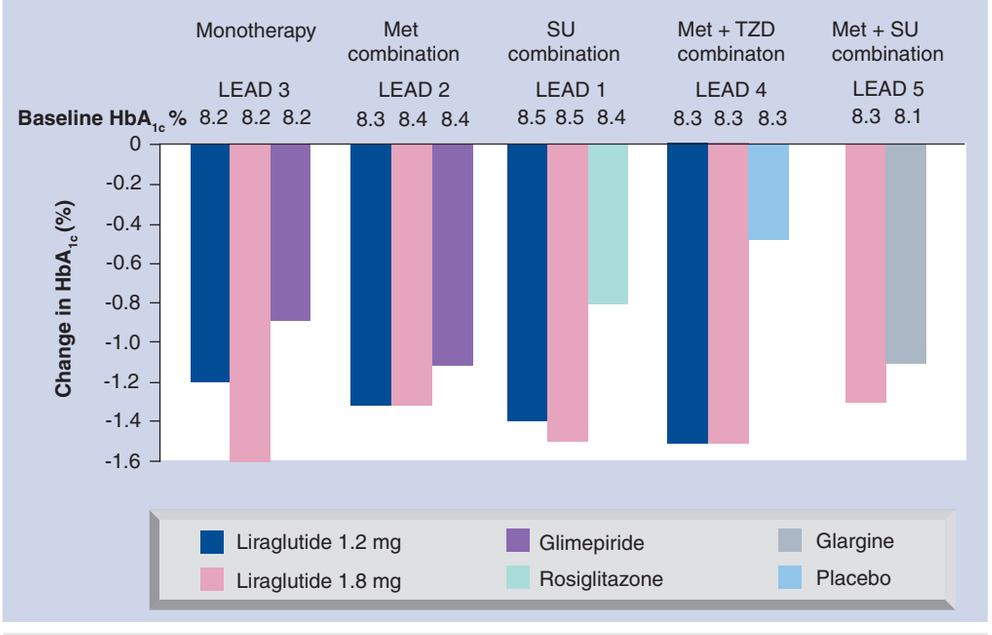

Figure 1. Reductions in HbA_{1c} in the LEAD Phase III trials using liraglutide. Data are change in HbA_{1c} from baseline for overall population (LEAD 4 and 5); add-on to diet and exercise failure (LEAD 3); or add-on to previous OAD monotherapy (LEAD 1 and 2).
*$p < 0.05$ vs comparator.
LEAD: Liraglutide Effect and Action in Diabetes; Met: Metformin; SU: Sulfonylurea; TZD: Rosiglitazone.

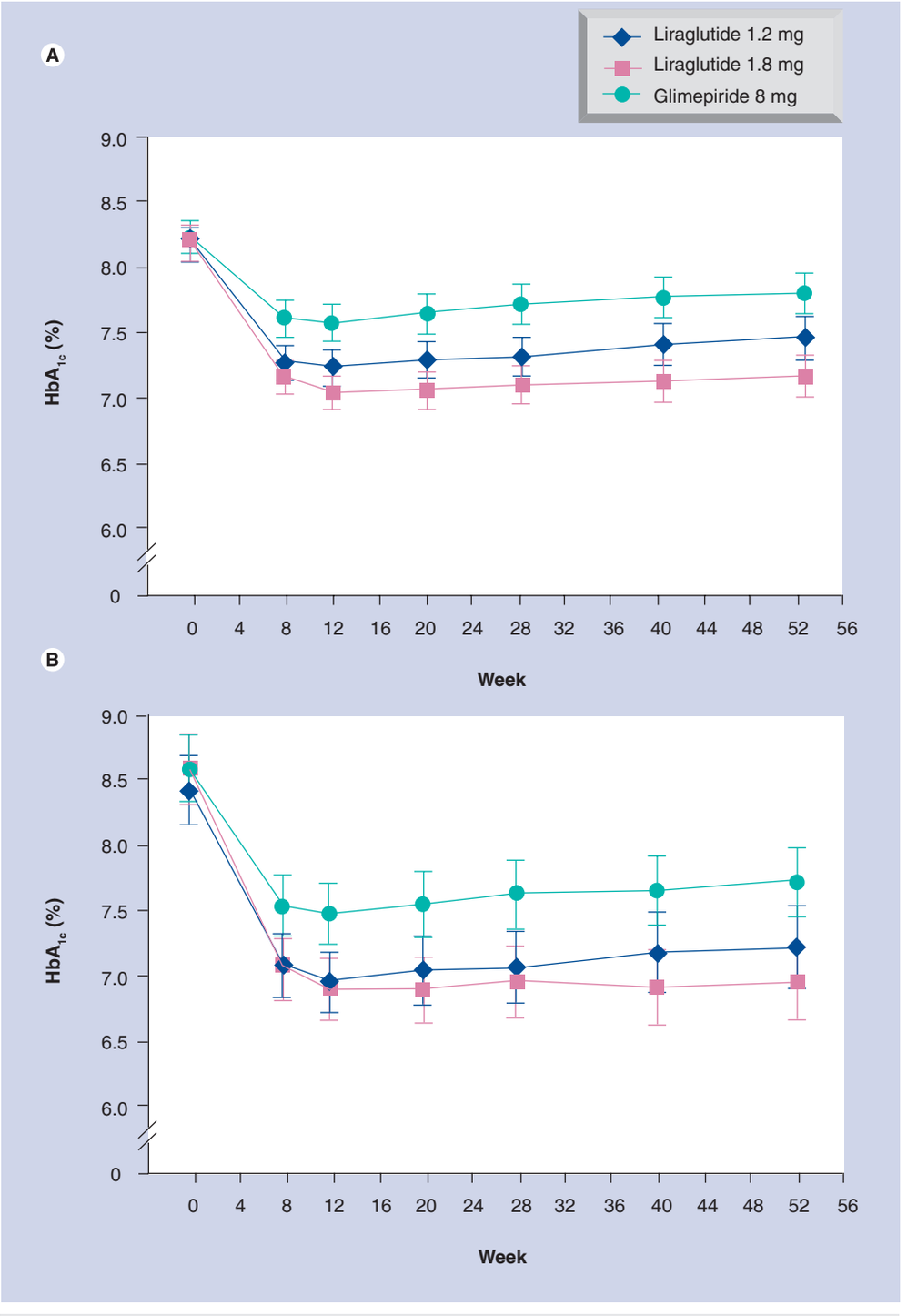

Figure 2. HbA_{1c} reductions with liraglutide monotherapy in the LEAD 3 study.
(A) All subjects; **(B)** Drug-naive subjects.
LEAD: Liraglutide Effect and Action in Diabetes.

randomized study (comparing liraglutide plus metformin with glimepiride plus metformin or metformin alone), liraglutide 1.8 mg plus metformin reduced HbA_{1c} by 1.0%, significantly more than metformin alone (change +0.09%, $p < 0.0001$) and comparable to the reduction with glimepiride/metformin [27]. Likewise, in the LEAD 1 study, liraglutide 1.8 mg plus glimepiride 4 mg reduced HbA_{1c} by 1.1%, significantly more than a 0.4% reduction with rosiglitazone plus glimepiride ($p < 0.0001$) and a 0.2% increase with glimepiride alone ($p < 0.0001$) [28].

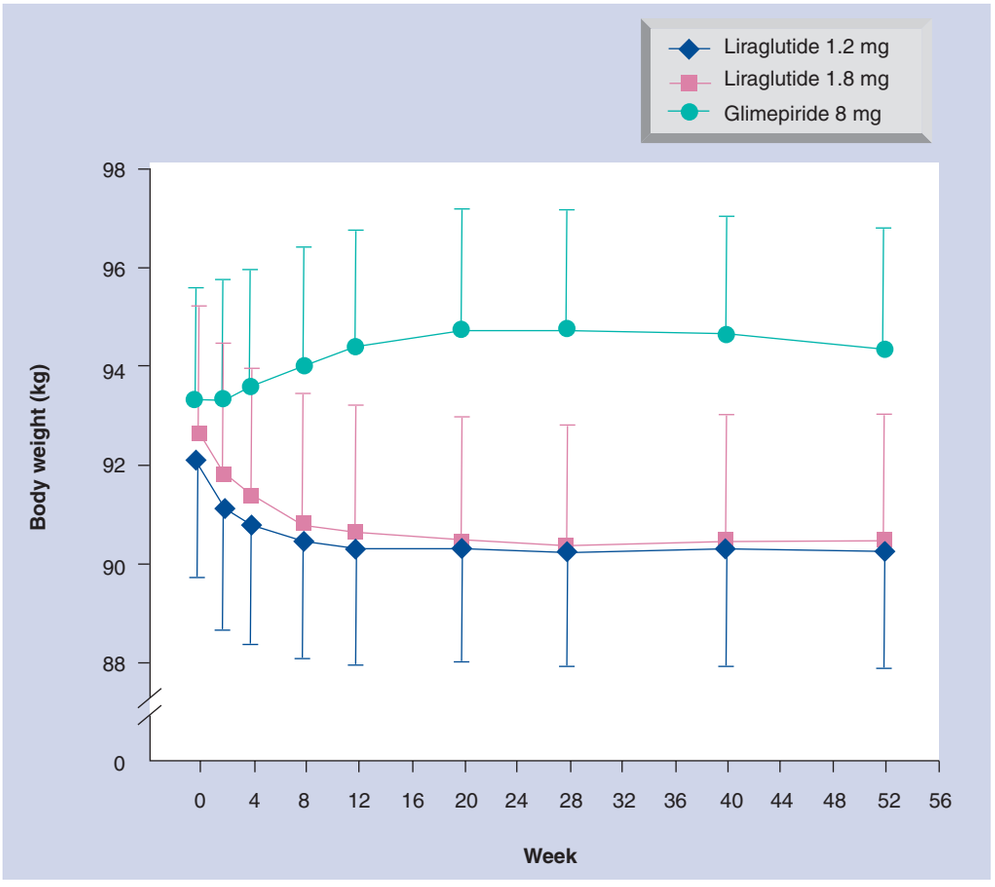

Figure 3. Changes in body weight in the LEAD 3 study.
LEAD: Liraglutide Effect and Action in Diabetes.

When used in combination with both metformin and rosiglitazone in the LEAD 4 study, liraglutide 1.8 mg achieved reductions in HbA_{1c} of 1.5%, while a reduction of 1.3% was achieved in combination with metformin and glimepiride in the LEAD 5 study [29,30]. In LEAD 4, the improvement with liraglutide was significantly greater than that with metformin/rosiglitazone alone (0.5%; $p < 0.0001$), while in LEAD 5 it was again significantly greater than metformin/glimepiride alone (1.3 vs 0.2%, $p < 0.001$), and also greater than insulin glargine plus metformin and glimepiride (1.1%; $p = 0.0015$).

A direct comparison between liraglutide and the insulin mimetic exenatide was reported from the open-labeled LEAD 6 study. Mean HbA_{1c} reduction was more pronounced with liraglutide treatment than with exenatide: -1.1 vs -0.8%, $p < 0.0001$ [31]. Accordingly, more subjects achieved an HbA_{1c} level of less than 7.0% with liraglutide treatment than with exenatide: 54 vs 43%; $p = 0.0015$. Differences were seen between the two agents regarding fasting glucose: fasting plasma glucose decreased by -1.6 mmol/l with liraglutide treatment and -0.6 mmol/l with exenatide ($p < 0.0001$).

Glucose reduction with liraglutide is associated with low levels of hypoglycemia, perhaps reflecting the glucose-dependent nature of its insulinotropic action. In the LEAD 3 monotherapy study, there were no major hypoglycemic events with liraglutide, and a rate of minor events less than 0.5 per patient per year ($p < 0.0001$ vs 2.0 events/patient/year with glimepiride) [26]. Minor hypoglycemic event rates were similarly low in the LEAD 1, 2 and 4 trials (0.5, 0.1 and 0.6 events/patient/year, respectively), and only slightly higher (1.2 events/patient/year) in the LEAD 5 study where liraglutide was used together with both sulfonylurea and metformin [27–30].

Body weight, blood pressure & cardiovascular risk markers

In addition to glucose control mediated by insulinotropic action, GLP-1-based therapies potentially have beneficial effects on body weight. These are likely to be mediated by central effects

on satiety, and also by a reduction in gastric emptying (so-called 'ileal brake' mechanism that contributes to the improved glucose control during GLP-1 treatment). Studies in animal models confirm the effect of liraglutide: obese candy-fed rats lost a mean 14.2 g after 12 weeks of liraglutide treatment, whereas control animals gained 24.3 g over the same period [32]. A further study in obese minipigs showed that a once-daily administration of liraglutide reduced feeding frequency, feeding duration and meal volume, which were reflected in a reduction of at least 60% in food intake and a significant reduction of 4.3 kg (baseline 93.7 kg) during the 7-week treatment period [33]. Withdrawal of liraglutide after 7 weeks of treatment resulted in a prompt increase in food intake and consequent weight gain.

Effects on body weight seen in preclinical data were confirmed in the LEAD studies: in LEAD 3, liraglutide 1.8 mg as monotherapy reduced mean body weight by 2.5 kg (baseline 93 kg), significantly different to a 1.1 kg increase in body weight with glimepiride ($p < 0.0001$; FIGURE 3) [26]. Liraglutide plus metformin in LEAD 2 resulted in a 2.8 kg weight reduction ($p < 0.0001$ vs 1.0 kg increase with glimepiride/metformin); liraglutide plus glimepiride in LEAD 1 yielded a more modest decrease of 0.2 kg that was nevertheless significantly different to a 2.1 kg increase with rosiglitazone/glimepiride [27,28]. Similarly, in combination with two oral antidiabetic agents, liraglutide 1.8 mg treatment yielded body weight reductions of around 2 kg (2.0 kg with liraglutide plus metformin and rosiglitazone in LEAD 4; 1.8 kg with liraglutide plus metformin and glimepiride in LEAD 5) [29,30]. The effect on body weight of liraglutide was independent of the presence and duration of gastrointestinal adverse events.

Cardiovascular risk markers were measured during the 14-week placebo-controlled Phase II study, and a significant decrease in plasminogen activator inhibitor 1 (PAI-1) and B-type natriuretic peptide (BNP) following treatment with liraglutide was found [34]. Interestingly, data from clinical trials show that when used for treatment of Type 2 diabetes, liraglutide is associated with a consistent, significant reduction in systolic blood pressure (SBP). Previously, reductions in SBP versus placebo of 5.2–7.9 mmHg were observed with liraglutide treatment [13]. Similarly, in the LEAD study program, SBP reductions of 2–6 mmHg were observed with liraglutide treatment. In LEAD 3, SBP fell by 3.6 mmHg with liraglutide 1.8 mg, significantly more than a 0.9 mmHg reduction in the glimepiride group ($p < 0.05$) [26]. The mechanism behind the effects of GLP-1-based therapy on the cardiovascular system is not completely elucidated and is currently being studied intensively.

β-cell function & disease progression

Sturis and colleagues studied the effects of liraglutide on β-cell function in Zucker diabetic fatty (ZDF) rats [35]. After 6 weeks of liraglutide treatment, animals showed an increase in insulin secretion, which contrasted with a decrease in vehicle-treated animals. This was accompanied by an increase in β-cell mass after 6 weeks of liraglutide treatment. Similar findings were reported by Rolin *et al.*, who found that liraglutide treatment significantly increased β-cell proliferation and β-cell mass in db/db mice [36]. A study by Bock and colleagues in nondiabetic rats failed to demonstrate increases in absolute β-cell mass, α-cell mass or total pancreatic mass; however, relative β-cell mass was initially increased as body weight decreased [37].

A study by Bregenholt and colleagues using isolated rat pancreatic islets suggested a protective and anti-apoptotic effect of liraglutide [38]. Liraglutide was effective in inhibiting cytokine and free-fatty-acid-induced apoptosis in islet cells in a dose-dependent manner. During the 14-week placebo-controlled Phase II study with liraglutide, β-cell function as measured by homeostasis model assessment (HOMA-B) increased significantly with liraglutide treatment vs placebo, with increases ranging from 75 to 134% in the three liraglutide groups (all $p < 0.0001$) [13]. Additionally, a dose-dependent, but nonsignificant, reduction in insulin resistance as measured by HOMA, together with significant improvements in the proinsulin:insulin ratio (changes of -0.15 to -0.19 vs placebo; all $p < 0.05$) were observed. As part of the same study, an insulin-modified frequently sampled glucose tolerance test and an arginine-stimulated insulin secretion test during hyperglycemic clamp were conducted [39]. Data from 28 subjects plus 12 healthy control subjects were available. It was found that treatment with liraglutide for 14 weeks markedly increased first-phase insulin secretion ($AUC_{insulin,\ 0–10\ min}$) by up to 118% versus placebo; $p = 0.02$. Second-phase insulin response ($AUC_{C\text{-}peptide,\ 19–40\ min}$) was also increased by up to 79% versus placebo; $p = 0.005$. Similarly, arginine-stimulated insulin secretion was increased following liraglutide treatment by up to 114% versus placebo; $p = 0.02$. No significant effect of liraglutide on insulin sensitivity was seen in this substudy; liraglutide also failed to restore

insulin responses in patients with Type 2 diabetes to levels in the healthy control subjects. In the current trial patients were evaluated during treatment with liraglutide, which was administered the evening before the experimental tests.

Tolerability & acceptability

The most frequently reported adverse effects during liraglutide treatment are gastrointestinal. In the 1-year LEAD 3 study of liraglutide monotherapy, 28–29% of patients in the liraglutide groups reported nausea, compared with 9% in the glimepiride group [26]. In total, 17 of 497 liraglutide-treated patients withdrew due to vomiting, nausea or diarrhea. In this, as in the other LEAD studies, the occurrence of nausea decreased over time, and fewer than 10% of liraglutide-treated subjects reported nausea by week 4. This is consistent with data obtained by Horowitz and colleagues, who used the Gastrointestinal System Rating Scale (GSRS), a validated self-reporting instrument (Likert scale from no [score 1] to very severe [score 7] discomfort. Scores were calculated as the mean value of evaluated item), to assess gastrointestinal tolerability during the 14-week Phase II study [40]. It was found that GSRS scores increased initially with liraglutide treatment, compared with placebo (from 1.4 to 2.0 vs from 1.0 to 1.6), but decreased towards baseline levels and were comparable in liraglutide and placebo groups at the end of the study (from 1.1 to 1.6 vs from 1.0 to 1.2, respectively). Data from LEAD 6 suggest that nausea may be less persistent with liraglutide treatment than with exenatide [31]. In this study, the nausea incidence with liraglutide treatment by week was 8% after 5 weeks, 4% after 10 weeks and 3% after 26 weeks, whereas the similar time-points showed 18, 13 and 10% with exenatide.

Liraglutide is administered as a once-daily subcutaneous injection using a pen device. Although the injectable nature of the therapy may prove less acceptable to patients than an oral therapy, the liraglutide regimen has the advantage of being once-daily. In studies with liraglutide, the final dose has been achieved by stepwise escalation in weekly increments of 0.6 mg; however, the dose does not thereafter need titration or adjustment (unlike the insulins).

As a non-native peptide, it is theoretically possible that liraglutide might induce an antibody response, which could act to limit its efficacy. The available evidence suggests that the frequency of antibody formation to liraglutide is comparatively low (up to 8–9%). So far, the antibodies do not seem to impair the effect of the compound.

Conclusion

Liraglutide seems to be safe and effective as a pharmacological therapy for Type 2 diabetes. Clinical trials demonstrate significant and sustained reductions in HbA_{1c}, fasting plasma glucose levels, postprandial glucose levels, weight and blood pressure, and improved β-cell function measured as HOMA and the proinsulin:insulin ratio. As most studies have been of short duration (<30 weeks), there are as yet no long-term data (>52 weeks) on the durability of glycemic control, weight loss and adverse effects. LEAD 2, LEAD 3 and LEAD 6 are currently in an extension phase, and answers to these questions will hence soon emerge.

The incretin-based therapies offer new options in the treatment of Type 2 diabetes. Whether incretin-based therapy will change the progression of Type 2 diabetes is unknown, but the results so far are promising. Their final role in the treatment of Type 2 diabetes remains to be clarified, and long-term trials with cardiovascular end points and safety data are also needed.

Future perspective

The incretin system is an area of great interest in the development of therapies for management of Type 2 diabetes. GLP-1 receptor agonists show significant promise, and may be widely used in the future as we demand agents with high efficacy and good tolerability. Meanwhile, expectations

Executive summary

- Liraglutide is a recombinant glucagon-like peptide-1 (GLP-1) analog with high homology to native GLP-1 and is suitable for once-daily administration.
- Clinical pharmacology studies with liraglutide have shown it to be capable of dose-dependent insulin secretion and 24-h glucose control.
- Liraglutide has been evaluated in Phase II and III studies as monotherapy and in combination with one or two oral antidiabetic agents.
- Liraglutide has achieved HbA_{1c} reductions of 1–1.5% in clinical studies with low risk of hypoglycemia.
- In clinical studies, liraglutide was also associated with body weight reductions of 1.8–2.8 kg and reductions in systolic blood pressure of 2–6 mmHg.
- Preclinical data suggest beneficial effects of liraglutide on β-cell mass and disease progression, supported by β-cell function data from clinical studies.

of diabetes therapies increasingly span effects beyond glycemia, and it is likely that future therapies will, like liraglutide, need to show beneficial effects on body weight, cardiovascular markers and β-cell function.

Financial & competing interests disclosure

Tina Vilsbøll has received fees for being a consultant, speaker and/or advisory board member for Eli Lilly, MannKind, MSD, Novartis and Novo Nordisk, and has received scientific funding from MSD. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Editorial assistance was provided by Cate Foster from Watermeadow Medical.

Bibliography

Papers of special note have been highlighted as:
- ▪ of interest
- ▪▪ of considerable interest

1 Nauck MA, Heimesaat MM, Behle K *et al.:* Effects of glucagon-like peptide 1 on counterregulatory hormone responses, cognitive functions, and insulin secretion during hyperinsulinemic, stepped hypoglycemic clamp experiments in healthy volunteers. *J. Clin. Endocrinol. Metab.* 87, 1239–1246 (2002).

2 Holz GHT, Kuhtreiber WM, Habener JF: Induction of glucose competence in pancreatic β-cells by gluagon like peptide 1(7–37). *Trans. Assoc. Am. Physician.* 105, 260–267 (1992).

3 MacDonald PE, Salpatek AM, Wheeler MB: Glucagon-like peptide-1 receptor activation antagonizes voltage-dependent repolarizing K^+ currents in β-cells: a possible glucose-dependent insulinotropic mechanism. *Diabetes* 51 (Suppl. 3), S443–S447 (2002).

4 Bode HP, Moorman B, Dabew R, Goke B: Glucagon-like peptide 1 elevates cytosolic calcium in pancreatic β-cells independently of protein kinase A. *Endocrinology* 140, 3919–3927 (1999).

5 Drucker DJ, Philippe J, Mojsov S, Chick WL, Habener JF: Glucagon-like peptide 1 stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. *Proc. Natl Acad. Sci. USA* 84, 3434–3438 (1987).

6 Fehman HC, Habener JF: Insulinotropic hormone glucagon-like peptide 1(7–37) stimulation of proinsulin gene expression and proinsulin biosynthesis in insulinoma β TC-1 cells. *Endocrinology* 130, 159–166 (1992).

7 Perfetti R, Zhou J, Doyle ME, Egan JM: Glucagon-like peptide 1 induces cell proliferation and pancreatic-duodenum homeobox-1 expression and increases endocrine cell mass in the pancreas of old, glucose-intolerant rats. *Endocrinology* 141, 4600–4605 (2000).

8 Stoffers DA, Kieffer TJ, Hussain MA *et al.*: Insulinotropic glucagon-like peptide 1 agonists stimulate expression of homeodomain protein IDX-1 and increase islet size in mouse pancreas. *Diabetes* 49, 741–748 (2000).

9 Farilla L, Bulotta A, Hirshberg B *et al.*: Glucagon-like peptide 1 inhibits cell apoptosis and improves glucose responsiveness of freshly isolated human islets. *Endocrinology* 144, 5149–5158 (2003).

10 Buteau J, El-Assaad W, Rhodes CJ, Rosenberg L, Joly E, Prentki M: Glucagon-like peptide 1 prevents β-cell glucolipotoxicity. *Diabetologia* 47, 806–815 (2004).

11 Flint A, Raben A, Astrup A, Holst JJ: Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. *J. Clin. Invest.* 101, 515–520 (1998).

12 Zander M, Madsbad S, Madsen JL, Holst JJ: Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and β-cell function in Type 2 diabetes: a parallel-group study. *Lancet* 359, 824–830 (2002).

▪ **Clinical proof-of-concept regarding glucagon-like peptide 1 (GLP-1) treatment of patients with Type 2 diabetes.**

13 Vilsbøll T, Zdravkovic M, Le-Thu T *et al.*: Liraglutide, a long-acting human glucagon-like peptide-1 analog, given as monotherapy significantly improves glycemic control and lowers body weight without risk of hypoglycemia in patients with Type 2 diabetes. *Diabetes Care* 30, 1608–1610 (2007).

14 Rachman J, Barrow BA, Levy JC, Turner RC: Near-normalisation of diurnal glucose concentrations by continuous administration of glucagon-like peptide 1 (GLP-1) in subjects with NIDDM. *Diabetologia* 40, 205–211 (1997).

15 Deacon CF, Nauck MA, Toft-Nielsen M, Pridal L, Willms B, Holst JJ: Both subcutaneously and intravenously administered glucagon-like peptide I are rapidly degraded from the NH2-terminus in Type II diabetic patients and in healthy subjects. *Diabetes* 44, 1126–1131 (1995).

▪ **First paper describing the degradation of GLP-1 by DPP-4.**

16 Deacon CF, Pridal L, Klarskov L, Olesen M, Holst JJ: Glucagon-like peptide 1 undergoes differential tissue-specific metabolism in the anesthetized pig. *Am. J. Physiol.* 271, E458–E464 (1996).

17 Bell GI, Santerre RF, Mullenbach GT: Hamster preproglucagon contains the sequence of glucagon and two related peptides. *Nature* 302, 716–718 (1983).

18 Knudsen LB, Nielsen OPF, Huusfeldt PO *et al.*: Potent derivatives of glucagon-like peptide-1 with pharmacokinetic properties suitable for once daily administration. *J. Med. Chem.* 43, 1664–1669 (2000).

19 Knudsen LB, Agerso H, Huusfeldt PO, Nielsen PF, Wilken M: Derivatives of glucagon-like peptide-1 suitable for once daily administration. *Diabetologia* 42 (Suppl. 1), A199 (1999).

20 Steensgaard DB, Thomsen JK, Olsen HB, Knudsen LB: The molecular basis for the delayed absorption of the once-daily human GLP-1 analogue, liraglutide. *Diabetes* 57 (Suppl. 1), A164 (2008).

21 Damholt B, Golor G, Wierich W, Pedersen P, Ekbloom M, Zdravkovic M: An open-label, parallel-group study investigating the effects of age and gender on the pharmacokinetics of the once-daily glucagon-like peptide-1 analogue liraglutide. *J. Clin. Pharmacol.* 46, 635–641 (2006).

22 Degn KB, Juhl CB, Sturis J *et al.*: One week's treatment with the long-acting glucagon like peptide 1 derivative liraglutide (NN2211) markedly improves 24-h glycemia and α- and β-cell function and reduced endogenous glucose release in patients with Type 2 diabetes. *Diabetes* 53, 1187–1194 (2004).

23 Nauck MA, El-Ouaghlidi A, Hompesch M, Jacobsen J, Elbrond B: No impairment of hypoglycemia counterregulation via glucagon with NN2211, a GLP-1 derivative, in subjects with Type 2-diabetes. *Diabetes* 52 (Suppl. 1), A128 (2003).

24 Nauck MA, Hompesch M, Filipczak R, Le-Tu DT, Zdravkovic M, Gomprecht J: Five weeks of treatment with the GLP-1 analogue liraglutide improves glycaemic control and lowers body weight in subjects with Type 2 diabetes. *Exp. Clin. Endocrinol. Diabetes* 114, 417–423 (2006).

25 Madsbad S, Schmitz O, Ranstam J, Jakobsen G, Matthews DR; the NN2211–1310 International Study Group: Improved glycemic control with no weight increase in patients with Type 2 diabetes after once-daily treatment with the long-acting glucagon-like peptide 1 analog liraglutide (NN2211): a 12-week, double-blind, randomized, controlled trial. *Diabetes Care* 27, 1335–1342 (2004).

26 Garber A, Henry R, Ratner R *et al.*; for the LEAD-3 (Mono) Study Group: Liraglutide versus glimepiride monotherapy for Type 2 diabetes (LEAD-3 Mono): a randomised, 52-week, phase III, double-blind, parallel-treatment trial. *Lancet* 373(9662), 473–481 (2009).

▪▪ **Monotherapy trial (52 weeks) with liraglutide compared with metformin.**

27 Nauck M, Frid A, Hermansen K *et al.*; for the LEAD-2 Metformin Study Group: Efficacy and safety comparison of liraglutide, glimepiride, and placebo, all in combination with metformin in Type 2 diabetes mellitus: the LEAD (liraglutide effect and action in diabetes)-2 study. *Diabetes Care* 32(1), 84–90 (2009).

▪▪ **The clinical efficacy of liraglutide versus glimepiride when added on to metformin.**

28 Marre M, Shaw J, Brandle M *et al.:* Liraglutide, a once-daily human GLP-1 analog, added to a sulfonylurea (SU) offers significantly better glycemic control and favourable weight change compared with rosiglitazone and SU combination therapy in subjects with Type 2 diabetes. *Diabetes* 57 (Suppl. 1), A4 (2008).

29 Zinman B, Gerich J, Buse J *et al.:* Effect of the GLP-1 analog liraglutide on glycemic control and weight reduction in patients on metformin and rosiglitazone: a randomized double-blind placebo-controlled trial. *Can. J. Diab.* 32(Suppl.) (Abstract 101) (2008).

30 Russell-Jones D, Vaag A, Schmitz O *et al.:* Significantly better glycemic control and weight reduction with liraglutide, a once-daily human GLP-1 analog, compared with insulin glargine: all as add-on to metformin and a sulfonylurea in Type 2 diabetes. *Diabetes* 57 (Suppl. 1), A159 (2008).

31 Blonde L, Rosenstock J, Sesti G *et al.:* Liraglutide: superior glycaemic control vs. exenatide when added to metformin and/or SU in Type 2 diabetes. *Can. J. Diabetes* 32(Suppl.) (Abstract 107) (2008).

32 Raun K, von Voss P, Gotfredsen C, Golozoubova V, Rolin B, Knudsen LB: Liraglutide, a long-acting glucagon-like peptide-1 analog, reduced body weight and food intake in obese candy-fed rats, whereas a dipeptidyl peptidase-IV inhibitor, vildagliptin, does not. *Diabetes* 56, 8–15 (2007).

33 Raun K, von Voss P, Knudsen LB: Liraglutide, a once-daily human glucagon-like peptide-1 analog, minimizes food intake in severely obese minipigs. *Obesity (Silver Springs)* 15, 1710–1716 (2007).

▪ **Preclinical trial with liraglutide demonstrating the effect of liraglutide on food intake.**

34 Courrèges J-P, Vilsbøll T, Zdravkovic M *et al.:* Beneficial effects of once-daily liraglutide, a human glucagon-like peptide-1 analogue, on cardiovascular risk biomarkers in patients with Type 2 diabetes. *Diabet. Med.* 25(9), 1129-1131 (2008).

35 Sturis J, Gotfredsen C, Rømer J *et al.:* GLP-1 derivative liraglutide in rats with β-cell deficiencies: influence of metabolic state on β-cell mass dynamics. *Br. J. Pharmacol.* 140, 123–132 (2003).

36 Rolin B, Larsen MO, Gotfredsen CF *et al.:* The long-acting GLP-1 derivative NN2211 ameliorates glycemia and increases β-cell mass in diabetic mice. *Am. J. Physiol. Endocrinol. Metab.* 283, E745–E752 (2002).

37 Bock T, Pakkenberg B, Buschard K: The endocrine pancreas in non-diabetic rats after short-term and long term treatment with the long-acting GLP-1 derivative NN2211. *APMIS* 111, 1117–1124 (2003).

38 Bregenholt S, Møldrup A, Blume N *et al.:* The long-acting glucacon-like peptide-1 analogue, liraglutide, inhibits β-cell apoptosis *in vitro*. *Biochem. Biophys. Res. Commun.* 330, 577–584 (2005).

39 Vilsbøll T, Brock B, Perrild H *et al.:* Liraglutide, a once-daily human GLP-1 analogue, improves pancreatic B-cell function and arginine-stimulated insulin secretion during hyperglycaemia in patents with Type 2 diabetes. *Diabet. Med.* 25, 152–156. (2008).

40 Horowitz M, Vilsbøll T, Zdravkovic M, Hammer M, Madsbad M: Patient-reported rating of gastrointestinal adverse effects during treatment of Type 2 diabetes with the once-daily human GLP-1 analogue, liraglutide. *Diabes. Obes. Metab.* 10, 593–596 (2008).

Website

101 CDC fact sheet 2005 (Accessed 18 November 2008). www.cdc.gov/diabetes/pubs/pdf/ndfs_2005.pdf