REVIEW PAPER

THE INTERNATIONAL JOURNAL OF CLINICAL PRACTICE

# Liraglutide: the therapeutic promise from animal models

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**Disclosures**
This article forms part of a supplement funded by Novo Nordisk.
LBK is an employee of Novo Nordisk. The author was part of the team that discovered and developed liraglutide. All patents naming the author as an inventor belong to Novo Nordisk; the author has no financial interest related to any patents. LBK has shares in Novo Nordisk as part of an employee offering programme.

## SUMMARY

**Aims:** To review the differences between the human glucagon-like peptide-1 (GLP-1) molecule and the analogue liraglutide, and to summarise key data from the liraglutide preclinical study programme showing the therapeutic promise of this new agent. **Key findings:** Liraglutide is a full agonist of the GLP-1 receptor and shares 97% of its amino acid sequence identity with human GLP-1. Unlike human GLP-1, however, liraglutide binds reversibly to serum albumin, and thus has increased resistance to enzymatic degradation and a longer half-life. In preclinical studies, liraglutide demonstrated good glycaemic control, mediated by the glucose-dependent stimulation of insulin and suppression of glucagon secretion and by delayed gastric emptying. Liraglutide also had positive effects on body weight, beta-cell preservation and mass, and cardiac function. **Conclusions:** The therapeutic promise of liraglutide is evident from preclinical data. Liraglutide showed the potential to provide good glycaemic control without increasing the risk of hypoglycaemia and, as with exenatide, but not dipeptidyl peptidase-4 inhibitors, to mediate weight loss. Although these benefits have subsequently been studied clinically, beta-cell mass can be directly studied only in animal models. In common with other incretin-based therapies, liraglutide showed the potential to modulate the progressive loss of beta-cell function that drives the continuing deterioration in glycaemic control in patients with type 2 diabetes. Body weight was lowered by a mechanism involving mainly lowered energy intake, but also potentially altered food preference and maintained energy expenditure despite weight loss.

## Introduction

As explained by Dr Unger in this supplement*, the therapeutic potential of incretin hormones for the treatment of type 2 diabetes is the subject of considerable ongoing research. Interest has focused on the incretin glucagon like peptide 1 (GLP 1) in particular and has resulted in two new classes of antihyperglycaemic agents: the dipeptidyl peptidase 4 (DPP 4) inhibitors and the GLP 1 receptor agonists. Liraglutide, which belongs to the latter class of agents, is one of only two commercially available GLP 1 receptor agonists. Liraglutide is approved for use in combination with selected oral agents (Europe and USA) and as monotherapy (USA and Japan).

In this first review of the supplement, differences between the human GLP 1 and liraglutide molecules are discussed and key data from the liraglutide preclinical programme are summarised. Preclinical work is a necessary precursor to human studies: assessment of toxicity in a preclinical setting is a legal requirement, but preclinical studies also gather important preliminary data on pharmacokinetics and pharmacodynamics. A large body of data in any preclinical programme is generated from *in vitro* investigations; however, normal animals and animal models of the disease state allow us to examine the effects of a new drug on the complex interplay of metabolic processes. This review focuses largely on the *in vivo* studies from the liraglutide preclinical programme, which demonstrate the therapeutic promise of liraglutide. These data continue to be relevant to the physician despite subsequent clinical trials, not least because animal models remain the only way to explore directly the mechanistic effects of therapy on different organs and tissues, such as beta cells. Animal studies are also essential to explore novel effects of a diabetes drug, such as a direct effect on cardiac function. Throughout this review, key findings for other incretin based therapies and GLP 1 are noted to provide context for the liraglutide data, and the

*Unger J. Liraglutide: can it make a difference in the treatment of type 2 diabetes? *Int J Clin Pract* 2010; **64** (Suppl. 167): 1–3.

reader is referred to existing reviews and/or key papers for more information. Subsequent articles in this supplement by Dr Schmidt, Drs Raskin and Mora, and Dr McGill consider the performance of liraglutide in clinical trials.

## The liraglutide molecule

Liraglutide is a full agonist of the GLP 1 receptor (1). The liraglutide and human GLP 1 molecules also share a 97% amino acid sequence identity. However, although the GLP 1 molecule is rapidly degraded in the body and has a half life of approximately 2 min following intravenous administration (2), the liraglutide molecule has a half life of 13 h following subcutaneous administration (3). Structurally, the molecules differ in only two respects (Figure 1). First, a C16 fatty acid chain (palmitic acid) is attached via a glutamic acid linker to lysine at position 26. Second, lysine is replaced with arginine at position 34, ensuring that the C16 side chain attaches only at position 26. The fatty acid chain allows reversible binding of liraglutide to albumin in the bloodstream, prolonging the action of liraglutide and increasing its resistance to degradation by the DPP 4 enzyme, and thus avoiding renal elimination. The fatty acid chain also allows liraglutide molecules to self associate into heptamers at the injection site, delaying absorption from the subcutis (4).

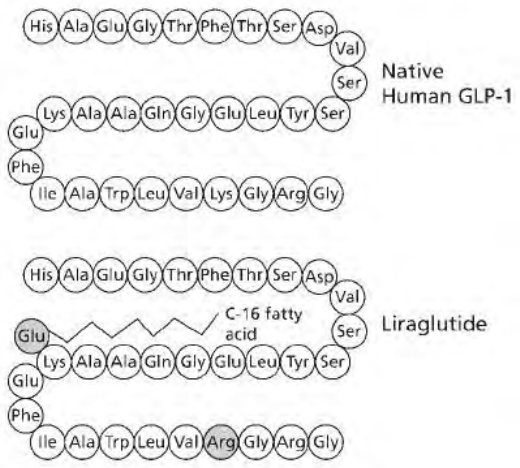


**Figure 1** Amino acid structure of human glucagon like peptide 1 (GLP 1) and liraglutide. Amino acids that are shaded in the liraglutide molecule differ from those in the GLP 1 molecule. (Reprinted from *Mol Cell Endocrinol*. Russell Jones D, Molecular, pharmacological and clinical aspects of liraglutide, a once daily GLP 1 analogue, pages 137 40, ©2009, with permission from Elsevier)

## Key findings from the liraglutide preclinical study programme

Preclinical data, particularly from animal models of diabetes and obesity, have revealed the considerable therapeutic potential of liraglutide. Beneficial effects are apparent in terms of glycaemic control, weight loss, beta cell regulation and cardiovascular function.

### Glycaemic control and hypoglycaemia

#### Background

The cornerstone of current antidiabetic therapy is aggressive control of hyperglycaemia. Achieving and maintaining control are commonly frustrated by treatment related increases in the risk of hypoglycaemia and in body weight and by the continued decline in beta cell function. GLP 1 based therapies are attractive therapeutic options because the stimulation of insulin secretion and suppression of glucagon release with human GLP 1 are glucose dependent (5), providing a degree of protection against hypoglycaemia. GLP 1 also impacts glycaemic control by slowing gastric emptying (6), thus reducing postprandial glucose excursions. The liraglutide preclinical programme examined its antihyperglycaemic and body weight lowering potential. Other available GLP 1 based therapies DPP 4 inhibitors (sitagliptin, saxagliptin and vildagliptin) that enhance the actions of the incretin hormones and the GLP 1 receptor agonist exenatide that mimics endogenous GLP 1 have shown glucose dependent antihyperglycaemic properties in preclinical trials (7 9) and are included as comparators in some of the studies discussed below.

#### Glycaemic control and hypoglycaemia with liraglutide

Liraglutide showed potent, long lasting, and both dose and glucose dependent antihyperglycaemic effects in numerous animal models of diabetes and obesity.

*Mouse models.* *Ob/ob* and *db/db* mice have increased body fat and insulin resistance compared with normal mice, with the severity of diabetes dependent on the age of the mouse. The increased body fat results from natural mutations in either the gene for leptin (*ob/ob* mice) or the leptin receptor (*db/db* mice).

Liraglutide showed a dose dependent and long lasting antihyperglycaemic effect in *ob/ob* mice (10). The mean area under the curve (AUC) for blood glucose, a measure of glucose excursion, was significantly lower after a single subcutaneous (s.c.) injection of liraglutide (30, 100, 300 or 1000 μg/kg) than

that after a single injection of vehicle. Moreover, 24 h after injection, blood glucose levels were still significantly lower than they were for controls. To investigate the effects of 24 h dosing in the same animal model, liraglutide was administered twice daily (bid) for 2 weeks. This contrasts with once daily dosing in humans because the half life of liraglutide is much longer in humans than in rodents. In this 24 h dosing study, blood glucose AUCs were significantly reduced with 100 μg/kg of liraglutide compared with vehicle at all assessment points (days 1, 8 and 15). Mean plasma insulin levels were also 60% higher in the liraglutide group than those in the vehicle group after 2 weeks.

Antihyperglycaemic effects were confirmed in *db/db* mice treated for 15 days with liraglutide (200 μg/kg bid s.c.) or vehicle (bid s.c.) (10). However, a shorter duration of effect was observed with the active comparator, exenatide (100 μg/kg bid s.c.). Furthermore, blood glucose levels in the exenatide group were similar to those in the vehicle group 10 12 h after dosing, whereas they were maintained throughout the 24 h monitoring period in the liraglutide group (Figure 2). The selected dose for exenatide was high in this study to facilitate a fair comparison. Normally, liraglutide is dosed 50 times higher than exenatide because most of the drug is bound to albumin.

*Rat models.* Liraglutide showed antihyperglycaemic effects in two rat models of diabetes: younger Zucker diabetic fatty (ZDF) rats showing insulin resistance without hyperglycaemia, and older, more overtly diabetic ZDF rats generally considered to be models for treatment resistant type 2 diabetes.

Younger ZDF rats were treated for 6 weeks with liraglutide (30 and 150 μg/kg bid s.c.) or vehicle (bid s.c.) (11). After glucose challenges on days 21 and 36, blood glucose levels were markedly lower for the liraglutide groups than for the vehicle group (Figure 3). Mean AUCs for blood glucose were significantly different among groups: lowest with the higher dose of liraglutide, intermediate with the lower dose of liraglutide and highest with vehicle. Dose dependent effects were also apparent for mean AUCs for plasma insulin after glucose challenge. In addition, although the average insulin AUCs increased by 66% between the first and second glucose challenges with the higher dose of liraglutide, AUCs decreased by 40% with vehicle. After 41 days of treatment, glucose and insulin AUCs for the higher dose liraglutide group were significantly lower than corresponding AUCs for the lower dose liraglutide and vehicle groups. Pair feeding, in which the daily food consumption for each ZDF rat in the liraglutide group was measured and then made available to a rat in the pair fed group (matched on the basis of initial body weight), showed that approximately 53% of the antihyperglycaemic effect on 24 h glucose profiles was mediated by a reduction in food intake.

Liraglutide retained some efficacy even in the older ZDF rats (12,13). In the 6 week study by Larsen et al., for example, a number of measures of plasma glucose were significantly reduced with liraglutide (200 μg/kg bid s.c.), but there were few differences between liraglutide and vehicle groups for measures of plasma insulin (12). Combination therapy with liraglutide and pioglitazone, however, synergistically

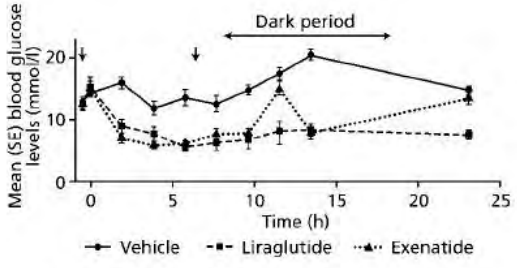


**Figure 2** Blood glucose levels in *db/db* mice receiving twice daily subcutaneous administration of either liraglutide 200 μg/kg, exenatide 100 μg/kg or vehicle (10). Data are for day 1, time 0 is at 9.00 AM and arrows indicate injection times; $n = 10$ per group. (Adapted from Rolin B et al. *Am J Physiol Endocrinol Metab* 2002; **283:** E745 52. Reproduced with permission; conveyed through Copyright Clearance Center, Inc.)

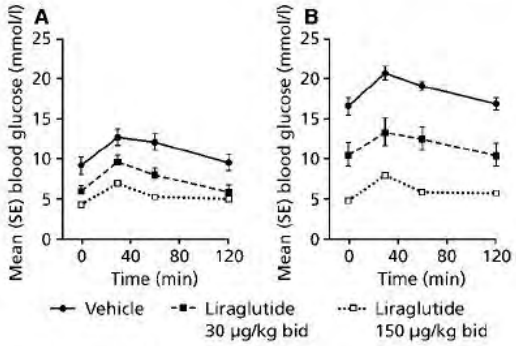


**Figure 3** Blood glucose levels after glucose challenge on (A) day 21 and (B) day 36 in fasted Zucker diabetic fatty rats (11). Rats were receiving subcutaneous injections of liraglutide (at one of two dose levels) or vehicle. Glucose was administered by gavage at time 0. Areas under the curve for blood glucose were significantly different for the three treatment groups ($p < 0.0005$ and 0.0002 for days 21 and 36, respectively). $n = 6$ per group. Bid, twice daily. (Adapted from Sturis J et al. *Br J Pharmacol* 2003; **140:** 123 32. Reproduced with permission from Wiley Interscience)

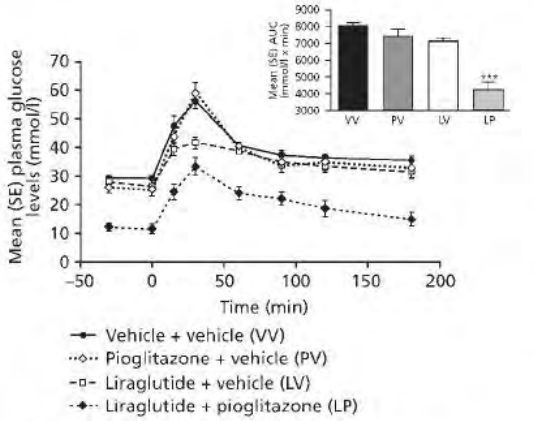


**Figure 4** Blood glucose levels and areas under the curve (AUC) in Zucker diabetic fatty rats after glucose challenge on day 42 (12). Rats received twice daily vehicle, liraglutide (200 μg/kg), pioglitazone (5 mg/kg) or a combination of liraglutide and pioglitazone. Glucose was administered at time 0. ***$p < 0.001$ vs. vehicle; $n = 10$ per group. (Adapted from Larsen PJ et al. Combination of the insulin sensitizer, pioglitazone, and the long acting GLP 1 human analogue, liraglutide, exerts potent synergistic glucose lowering efficacy in severely diabetic ZDF rats. *Diabetes Obes Metab* © 2008, with permission from Wiley Blackwell)

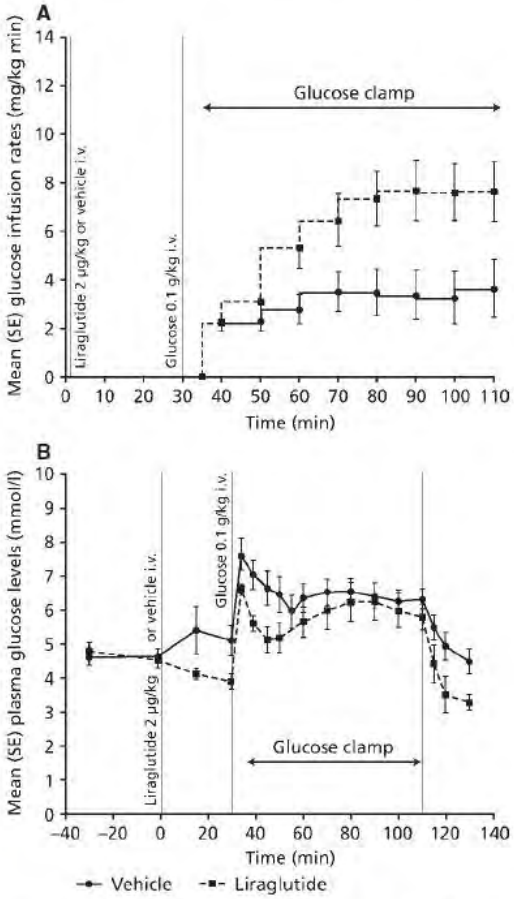


**Figure 5** (A) Glucose infusion rates during and (B) plasma glucose levels before, during and after a hyperglycaemic clamp in fasted minipigs (14). Minipigs received either liraglutide or glucose intravenously (i.v.) before the clamp. Animals treated with liraglutide required a significantly greater glucose infusion rate ($p < 0.005$), and still had slightly lower plasma glucose levels, than animals receiving vehicle. $n = 6$. (Adapted from Ribel U et al. *Eur J Pharmacol* 2002; **451:** 217 25. Reprinted from *Eur J Pharmacol.*, Ribel U et al., NN2211: a long acting glucagon like peptide 1 derivative with anti diabetic effects in glucose intolerant pigs, pages 217 25, © 2002, with permission from Elsevier)

improved glycaemic control even at week 6 (Figure 4).

*Minipig model.* Studies of the efficacy of liraglutide were conducted in minipigs made diabetic with streptozotocin (14). Minipigs and humans have simi lar skin, and injectable compounds therefore display similar pharmacokinetics. Short term investigations showed that liraglutide has a glucose dependent anti hyperglycaemic effect, whereas longer term studies showed that liraglutide reduces gastric emptying and improves glucose tolerance and insulin sensitivity (14).

A group of minipigs receiving intravenous (i.v.) liraglutide (2 μg/kg) required almost a 200% increase in the glucose infusion rate compared with the vehicle group during a hyperglycaemic clamp, and even then had slightly lower blood glucose levels (Figure 5). Mean AUCs for plasma insulin were markedly higher and mean AUCs for plasma gluca gon markedly lower during the clamp for the liraglu tide group than for the vehicle group. Moreover, data from animals under a hyperglycaemic clamp and animals receiving a low dose glucose infusion showed that the correlations between insulin and glucagon values were linear for minipigs receiving liraglutide or vehicle. However, the steeper slope with liraglutide indicated that responses were glucose dependent. Importantly, at low glucose levels, plasma glucagon concentrations rose, contributing to the maintenance of normoglycaemia.

In a chronic dosing study, minipigs received either liraglutide [3.3 μg/kg s.c. once daily (qd)] or vehicle for 4 weeks (14). The liraglutide group had signifi cantly reduced gastric emptying (assessed as the AUC for paracetamol) and improved glucose tolerance at 2 and 4 weeks compared with the vehicle group. Fur thermore, insulin sensitivity (assessed as glucose to insulin ratio) improved for the liraglutide group dur ing the study, but was unchanged in the vehicle group.

## Weight loss

### Background

A large proportion of patients with type 2 diabetes are overweight or obese, and it is estimated that even a modest weight loss of 1 kg will result in decreases in fasting plasma glucose of 0.2 mmol/l (15). In humans, GLP 1 enhances satiety, resulting in weight loss (16,17). The mechanism for GLP 1 induced low ering of body weight may involve both gastric emp tying and an effect in the brain.

Importantly, preclinical data also reveal key differ ences between GLP 1 receptor agonists and DPP 4 inhibitors in terms of their effects on body weight. Only GLP 1 receptor agonists mediate reduced food intake and weight loss (7,18,19). This corresponds with clinical data showing weight loss with the GLP 1 receptor agonists, but weight neutrality with the DPP 4 inhibitors*. Preclinical data further show that although the effects of GLP 1, liraglutide and exenatide on insulin release are glucose dependent (see earlier), their effects on appetite regulation are not (18).

### Liraglutide in normal rats and rat models

The effects of liraglutide on food intake and body weight were investigated in normal rats. Investiga tions were also undertaken in a number of rat mod els: rats made obese by neonatal exposure to monosodium glutamate; female rats showing increased food intake, weight and fat gain, and impaired mean glucose tolerance after receiving olanzapine; candy fed rats showing increased calorie consumption; ZDF rats with insulin resistance (11,12,18 21). These studies showed that liraglutide reduced food intake, body weight, fat mass and glu cose tolerance. Body weight was lowered by a mecha nism involving mainly lowered energy intake, but also potentially altered food preference and main tained energy expenditure despite weight loss. The findings are exemplified below by the studies in candy fed and ZDF rats.

The effects of liraglutide and vildagliptin on body weight and food intake were compared in candy fed rats (18). First, the rats were fed chow and supple mentary candy, which resulted in increased weight (mostly attributable to an increase in fat mass) and a slight increase in feeding associated energy expendi ture. Over the following 12 weeks, mean body weights returned to normal in rats who received lira glutide (0.2 mg/kg bid s.c.) alongside supplementary candy and also in rats reverting to a chow only diet. Most of the weight loss in the liraglutide group was attributable to a relative decrease in fat mass, assessed by dual energy X ray absorptiometry. As expected, there was no increase in plasma insulin levels in the liraglutide group to mediate the weight loss, as the effect of liraglutide on insulin release is glucose dependent (see earlier) and these rats were normoglycaemic. Instead, the weight loss seems likely to have resulted from the decreased calorie intake, with a shift in favour of chow over candy, and raised energy expenditure. In contrast, the vildagliptin (10 mg/kg bid orally) + supplementary candy group gained weight over the 12 week period, as did the group continuing to receive no treatment + supple mentary candy. Furthermore, whole body fat masses at end point were significantly higher in these two groups compared with the liraglutide + candy group.

In a 6 week study of ZDF rats, animals receiving liraglutide (150 μg/kg bid s.c.) had a significantly reduced mean daily food intake compared with rats receiving vehicle (11). The increase in mean body weight was also significantly less for the liraglutide group compared with the vehicle group after 10 days. Although the between group difference had disappeared by day 42, this reflected the reduced loss of calories attributable to glycosuria in the liraglutide group.

In older and more overtly diabetic ZDF rats, treat ment with liraglutide (200 μg/kg bid s.c.) for 6 weeks was associated with significantly decreased daily food intake and body weight and some reduc tion in fat depots compared with vehicle (12).

### Liraglutide in a minipig model

Weight loss studies were also conducted in minipigs. Obesity and feeding behaviour in these animals more closely resemble those of humans than those of rodents. Pigs mainly eat in meals during the light period and do not eat in the dark period; they also show increases in body fat that resemble humans, which rodents do not unless they have a severe monogenic form of obesity. Liraglutide reduced food intake and body weight in severely obese, hyper phagic minipigs (22). Mean food intake was greatly reduced during 7 weeks of treatment with liraglutide (7 μg/kg qd s.c.) compared with pre and post treat ment periods (Figure 6A) and mean body weights were generally stable before treatment, but decreased during treatment (Figure 6B).

## Beta-cell regulation

### Background

The progressive loss of beta cell function ultimately drives the continuing deterioration in glycaemic

*McGill JB. Liraglutide: effects beyond glycaemic control in diabetes treatment. *Int J Clin Pract* 2010; **64** (Suppl. 167): 28 34.

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