REVIEW PAPER

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Liraglutide: the therapeutic promise from animal models

L. B. Knudsen

Novo Nordisk A/S, Novo Nordisk Park, DK 2760, Måløv, Denmark

Correspondence to:
Lotte Bjerre Knudsen, Department Biology and Pharmacology Mgt, Novo Nordisk A/S, Novo Nordisk Park, Måløv, DK 2760 Denmark
Tel.: +45 444 3 4788
Fax: +45 3075 4788
Email: lbkn@novonordisk.com

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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.

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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 approx imately 2 min following intravenous administration (2), the liraglutide molecule has a half life of 13 h following subcutaneous administration (3). Struc turally, 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 argi nine 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 degra dation 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).

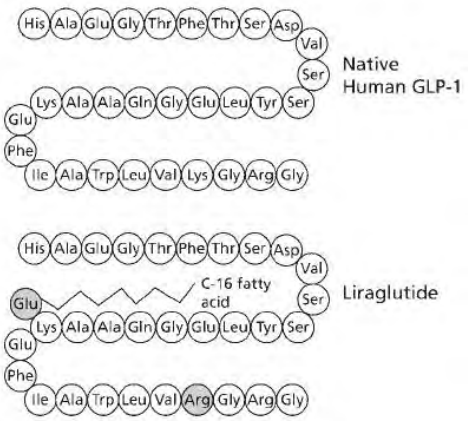

Figure 1 Amino acid structure of human glucagon like peptide 1 (GLP 1) and liraglutide. Amino acids that are shaded in the liraglutide molecule differ from those in the GLP 1 molecule. (Reprinted from *Mol Cell Endocrinol*. Russell Jones D, Molecular, pharmacological and clinical aspects of liraglutide, 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 hypoglyca emia and in body weight and by the continued decline in beta cell function. GLP 1 based therapies are attractive therapeutic options because the stim ulation 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 glycae mic control by slowing gastric emptying (6), thus reducing postprandial glucose excursions. The lira glutide preclinical programme examined its antihy perglycaemic 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 preclin ical 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 signifi cantly lower after a single subcutaneous (s.c.) injec tion of liraglutide (30, 100, 300 or 1000 μg/kg) than

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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

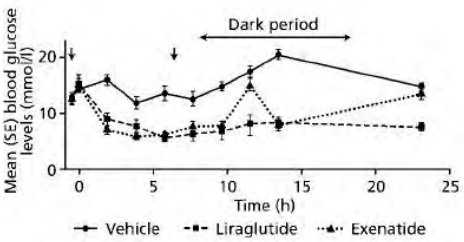

Figure 2 Blood glucose levels in *db/db* mice receiving twice daily subcutaneous administration of either liraglutide 200 μg/kg, exenatide 100 μg/kg or vehicle (10). Data are for day 1, time 0 is at 9.00 AM and arrows indicate injection times; $n = 10$ per group. (Adapted from Rolin B et al. *Am J Physiol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

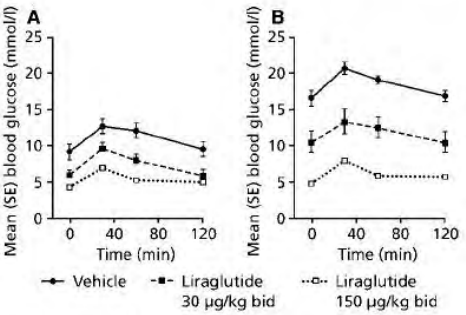

Figure 3 Blood glucose levels after glucose challenge on (A) day 21 and (B) day 36 in fasted Zucker diabetic fatty rats (11). Rats were receiving subcutaneous injections of liraglutide (at one of two dose levels) or vehicle. Glucose was administered by gavage at time 0. Areas under the curve for blood 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)

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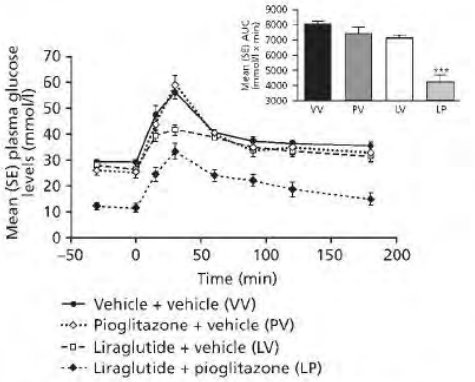

Figure 4 Blood glucose levels and areas under the curve (AUC) in Zucker diabetic fatty rats after glucose challenge on day 42 (12). Rats received twice daily vehicle, liraglutide (200 μg/kg), pioglitazone (5 mg/kg) or a combination of liraglutide and pioglitazone. Glucose was administered at time 0. ***$p < 0.001$ 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)

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 similar 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 glucagon markedly lower during the clamp for the liraglutide 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

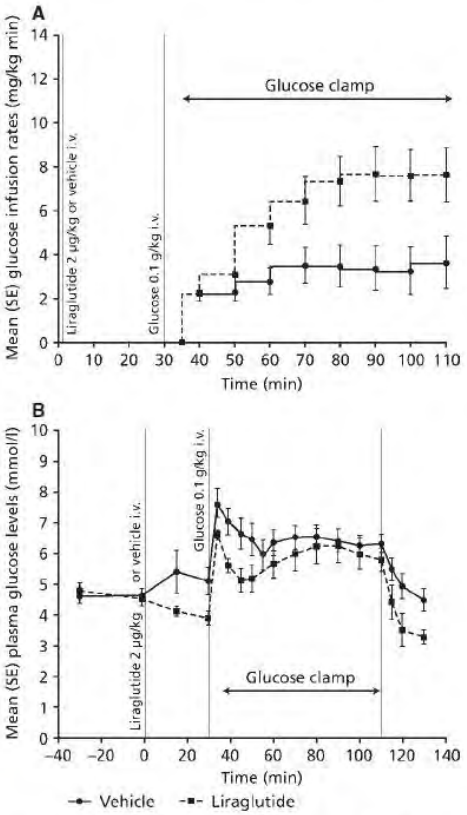

Figure 5 (A) Glucose infusion rates during and (B) plasma glucose levels before, during and after a hyperglycaemic clamp in fasted minipigs (14). Minipigs received either liraglutide or glucose intravenously (i.v.) before the clamp. Animals treated with liraglutide required a significantly greater glucose 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)

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 significantly reduced gastric emptying (assessed as the AUC for paracetamol) and improved glucose tolerance at 2 and 4 weeks compared with the vehicle group. Furthermore, insulin sensitivity (assessed as glucose to insulin ratio) improved for the liraglutide group during the study, but was unchanged in the vehicle group.

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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 lowering of body weight may involve both gastric emptying and an effect in the brain.

Importantly, preclinical data also reveal key differences 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. Investigations were also undertaken in a number of rat models: 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 glucose tolerance. 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. 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 supplementary candy, which resulted in increased weight (mostly attributable to an increase in fat mass) and a slight increase in feeding associated energy expenditure. Over the following 12 weeks, mean body weights returned to normal in rats who received liraglutide (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 + supplementary 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, treatment with liraglutide (200 μg/kg bid s.c.) for 6 weeks was associated with significantly decreased daily food intake and body weight and some reduction 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, hyperphagic 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 treatment 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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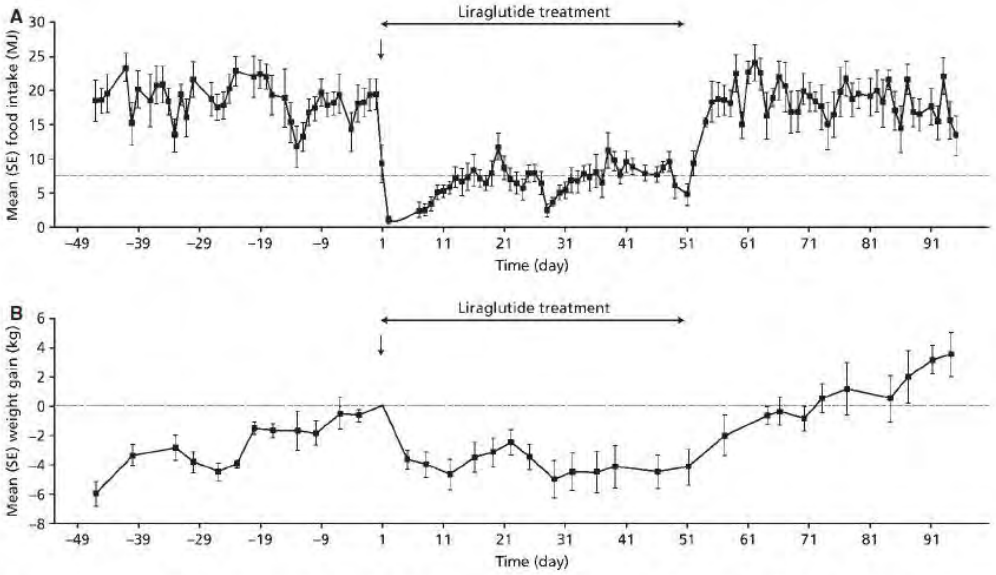

Figure 6 (A) Mean food intake and (B) weight gain in severely obese, hyperphagic minipigs before, during and after 7 weeks of treatment with liraglutide (7 μg/kg once daily administered subcutaneously) (22). The horizontal dotted lines represent the mean food intake (A) for the maintenance of normal body weight and the body weight (B) at the start of treatment; $n = 6$. (Figure previously published in Raun K et al. *Obesity* 2007; **15:** 1710 6)

control and thus repeated therapy failure. In the absence of reliable non invasive measures of beta cell regulation in humans, our understanding of the effects of GLP 1 and related therapies are necessarily shaped by *in vitro* and preclinical *in vivo* investigations and by surrogate clinical measures. Research with GLP 1 indicates that it has a positive influence on beta cell mass via a combination of increased beta cell neogenesis and proliferation, and reduced beta cell apoptosis (23). Preclinical studies with exenatide suggest it has a similar profile of positive effects (7).

Beta-cell regulation with liraglutide

Liraglutide increases beta cell mass in hyperglycaemic animals, but has temporary or no effects in normoglycaemic animals (10,11,20). Liraglutide has also been shown to have an inhibitory effect on beta cell apoptosis after syngeneic mouse transplants (24). Both the stimulatory and inhibitory effects were apparent in *in vitro* studies (25 27).

Mouse models. In *ob/ob* mice, liraglutide treatment (100 μg/kg bid s.c. for 15 days) had no effect on beta cell mass, but there was a tendency for beta cell proliferation to be increased in the liraglutide group compared with the vehicle group (10). In *db/db* mice, both the mean beta cell proliferation rate and the mean beta cell mass were significantly increased in the liraglutide group (200 μg/kg bid s.c.) compared with the vehicle group after 15 days of treatment (10) (Figure 7). In contrast, only the beta cell proliferation rate was significantly greater in the exenatide group (100 μg/kg bid s.c.), most likely illustrating the shorter duration of action.

Liraglutide (200 μg/kg s.c. bid) has also been administered after a marginal mass syngeneic islet transplant in streptozotocin induced diabetic BALB/c mice. The rate of beta cell apoptosis in the graft

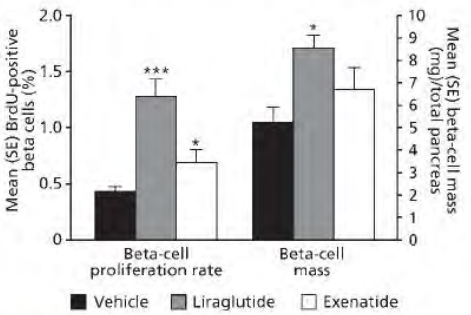

Figure 7 Beta cell proliferation rate and beta cell mass after 15 days of twice daily subcutaneous injection with liraglutide (200 μg/kg), exenatide (100 μg/kg) or vehicle in female *db/db* mice (10). (Adapted from Rolin B et al. *Am J Physiol Endocrinol Metab* 2002; **283:** E745 52. Reproduced with permission; conveyed through Copyright Clearance Center, Inc. $^*p < 0.05$; $^{***}p < 0.001$. BrdU, bromodeoxyuridine. $n = 10$ per group)

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bearing kidneys of animals treated with liraglutide was significantly lower than that in control mice after 48 h (24). Liraglutide was also associated with a reduced time to achieve normoglycaemia and better long term glycaemic control than vehicle (24), suggesting therapeutic potential for liraglutide in islet transplantation.

Normal rats and rat models. In two 6 week studies of ZDF rats with insulin resistance and beta cell defects, there were no significant differences between liraglutide (30 or 150 μg/kg bid s.c.) and vehicle groups for beta cell proliferation (11). However, beta cell mass was significantly greater for two of the three liraglutide groups compared with respective vehicle controls. Importantly, in non diabetic rats, the mean beta cell mass was significantly greater in the group treated with liraglutide 200 μg/kg bid s.c. for 1 week than in the group receiving vehicle, but beta cell mass was normalised after a total of 6 weeks of treatment (20), suggesting that effects in normoglycaemic animals are temporary.

In vitro work. The stimulatory effect of liraglutide was demonstrated in a study of primary monolayer cultures of newborn rat islet cells incubated with GLP 1, glucose dependent insulinotropic polypeptide or liraglutide for 24 h (25). The incorporation of bromodeoxyuridine, a measure of DNA synthesis, was 50 80% above the basal rate in all cases. A GLP 1 receptor mechanism was implicated as the effect was blocked with the antagonist exendin(9 39).

An inhibitory effect on beta cell apoptosis *in vitro* was apparent in two separate studies (26,27). Liraglutide had a dose dependent protective effect on cytokine and free fatty acid mediated beta cell apoptosis in primary neonatal rat islets, and the effect was significantly better for liraglutide than native GLP 1 (26). Again, a GLP 1 receptor mechanism was implicated as the effect was blocked with exendin(9 39). Liraglutide also reduced beta cell apoptosis in porcine islets in culture prior to transplantation (27). The rate of beta cell apoptosis at 24 h in cultures incubated with liraglutide was halved compared with cultures incubated with vehicle.

Cardiovascular function

Background

As GLP 1 receptors are expressed in the heart, vascular smooth muscle and regions of the central nervous system that regulate the cardiovascular system, there is clinical interest in GLP 1 based therapies for patients with diabetes who are in poor cardiovascular health. However, there are currently few preclinical studies into the effects of GLP 1, and the mechanisms underlying the effects are poorly understood. To date, preclinical studies have shown that GLP 1 receptor agonists have cardioprotective effects and reduce blood pressure in hypertensive rats (23,28 31). Studies also show that both GLP 1 and a metabolite, GLP 1(9 36), mediate effects via GLP 1 receptors and also independently of them (32).

Cardioprotective effects with liraglutide

The most extensive preclinical investigation with liraglutide has been conducted in a mouse model of myocardial infarction (33). Pretreatment with liraglutide [200 μg/kg intraperitoneally (i.p.)] for 7 days increased cardiomyocyte survival and improved cardiac function compared with controls. These effects were not an indirect effect of weight loss, as increased survival was also apparent at a weight neutral lower dose (75 μg/kg i.p.).

Conclusions

An extensive series of preclinical studies have revealed considerable potential benefits for liraglutide in the treatment of type 2 diabetes. Good glycaemic control was mediated by the glucose dependent stimulation of insulin and suppression of glucagon secretions, and also by delayed gastric emptying. Moreover, liraglutide showed positive effects on beta cell preservation and mass and on cardiac function. Although these benefits were also apparent with other incretin based therapies, exenatide and liraglutide stand apart in offering the potential to reduce body weight, a key concern in the management of type 2 diabetes. Of these two agents, liraglutide is longer acting than exenatide and is dosed only once daily compared with twice daily for exenatide. Clinical data indicate that liraglutide has a better glycaemic efficacy than exenatide despite the once daily dosing (see Raskin and Mora in this supplement*).

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Author contributions

The outline and drafts were developed in conjunction with the author, who approved the final version before submission.

*Raskin P, Mora PF. Glycaemic control with liraglutide: the phase 3 trial programme. *Int J Clin Pract* 2010; **64** (Suppl. 167): 21 7.

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References

1 Knudsen LB, Nielsen PF, Huusfeldt PO et al. Potent derivatives of glucagon-like peptide-1 with pharmacokinetic properties suitable for once daily administration. *J Med Chem* 2000; **43**: 1664–9.

2 Vilsbøll T, Agersø H, Krarup T, Holst JJ. Similar elimination rates of glucagon-like peptide-1 in obese type 2 diabetic patients and healthy subjects. *J Clin Endocrinol Metab* 2003; **88**: 220–4.

3 Agersø H, Jensen LB, Elbrønd B, Rolan P, Zdravkovic M. The pharmacokinetics, pharmacodynamics, safety and tolerability of NN2211, a new long-acting GLP-1 derivative, in healthy men. *Diabetologia* 2002; **45**: 195–202.

4 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* 2008; **57**(Suppl 1): A164.

5 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* 2002; **87**: 1239–46.

6 Nauck MA, Niedereichholz U, Ettler R et al. Glucagon-like peptide-1 inhibition of gastric-emptying outweighs its insulinotropic effects in healthy humans. *Am J Physiol* 1997; **273**: E981–8.

7 Nielsen LL, Young AA, Parkes DG. Pharmacology of exenatide (synthetic exendin-4): a potential therapeutic for improved glycemic control of type 2 diabetes. *Regul Pept* 2004; **117**: 77–88.

8 Mu J, Woods J, Zhou YP et al. Chronic inhibition of dipeptidyl peptidase-4 with a sitagliptin analog preserves pancreatic beta-cell mass and function in a rodent model of type 2 diabetes. *Diabetes* 2006; **55**: 1695–704.

9 Winzell MS, Ahrén B. The high-fat diet-fed mouse. A model for studying mechanisms and treatment of impaired glucose tolerance and type 2 diabetes. *Diabetes* 2004; **53**(Suppl 3): S215–9.

10 Rolin B, Larsen MO, Gotfredsen CF et al. The long-acting GLP-1 derivative NN2211 ameliorates glycemia and increases beta-cell mass in diabetic mice. *Am J Physiol Endocrinol Metab* 2002; **283**: E745–52.

11 Sturis J, Gotfredsen CF, Rømer J et al. GLP-1 derivative liraglutide in rats with beta-cell deficiencies: influence of metabolic state on beta-cell mass dynamics. *Br J Pharmacol* 2003; **140**: 123–32.

12 Larsen PJ, Wulff EM, Gotfredsen CF et al. Combination of the insulin sensitizer, pioglitazone, and the long-acting GLP-1 human analog, liraglutide, exerts potent synergistic glucose-lowering efficacy in severely diabetic ZDF rats. *Diabetes Obes Metab* 2008; **10**: 301–11.

13 Brand CL, Galsgaard ED, Tornehave D et al. Synergistic effect of the human GLP-1 analogue liraglutide and a dual PPARα/γ agonist on glycaemic control in Zucker diabetic fatty rats. *Diabetes Obes Metab* 2009; **11**: 795–803.

14 Ribel U, Larsen MO, Rolin B et al. NN2211: a long-acting glucagon-like peptide-1 derivative with anti-diabetic effects in glucose-intolerant pigs. *Eur J Pharmacol* 2002; **451**: 217–25.

15 Anderson JW, Konz EC. Obesity and disease management: effects of weight loss on comorbid conditions. *Obes Res* 2001; **9**(Suppl 4): 326–34S.

16 Flint A, Raben A, Astrup A, Holst JJ. Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. *J Clin Invest* 1998; **101**: 515–20.

17 Zander M, Madsbad S, Madsen JL, Holst JJ. Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. *Lancet* 2002; **359**: 824–30.

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

19 Larsen PJ, Fledelius C, Knudsen LB, Tang-Christensen M. Systemic administration of the long-acting GLP-1 derivative NN2211 induces lasting and reversible weight loss in both normal and obese rats. *Diabetes* 2001; **50**: 2530–9.

20 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* 2003; **111**: 1117–24.

21 Lykkegaard K, Larsen PJ, Vrang N, Bock C, Bock T, Knudsen LB. The once-daily human GLP-1 analog, liraglutide, reduces olanzapine-induced weight gain and glucose intolerance. *Schizophr Res* 2008; **103**: 94–103.

22 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 Spring)* 2007; **15**: 1710–6.

23 Baggio LL, Drucker DJ. Biology of incretins: GLP-1 and GIP. *Gastroenterology* 2007; **132**: 2131–57.

24 Merani S, Truong W, Emamaullee JA, Toso C, Knudsen LB, Shapiro AMJ. Liraglutide, a long-acting human glucagon-like peptide 1 analog, improves glucose homeostasis in marginal mass islet transplantation in mice. *Endocrinology* 2008; **149**: 4322–8.

25 Friedrichsen BN, Neubauer N, Lee YC et al. Stimulation of pancreatic β-cell replication by incretins involves transcriptional induction of cyclin D1 via multiple signalling pathways. *J Endocrinol* 2006; **188**: 481–92.

26 Bregenholt S, Møldrup A, Blume N et al. The long-acting glucagon-like peptide-1 analogue, liraglutide, inhibits beta-cell apoptosis *in vitro*. *Biochem Biophys Res Commun* 2005; **330**: 577–84.

27 Emamaullee JA, Merani S, Toso C et al. Porcine marginal mass islet autografts resist metabolic failure over time and are enhanced by early treatment with liraglutide. *Endocrinology* 2009; **150**: 2145–52.

28 Mafong DD, Henry RR. The role of incretins in cardiovascular control. *Curr Hypertens Rep* 2009; **11**: 18–22.

29 Sonne DP, Engstrøm T, Treiman M. Protective effects of GLP-1 analogues exendin-4 and GLP-1(9–36) amide against ischemia–reperfusion injury in rat heart. *Regul Pept* 2008; **146**: 243–9.

30 Laugero KD, Stonehouse AH, Guss S, Landry J, Vu C, Parkes DG. Exenatide improves hypertension in a rat model of the metabolic syndrome. *Metab Syndr Relat Disord* 2009; **7**: 327–34.

31 Yu M, Moreno C, Hoagland KM et al. Antihypertensive effect of glucagon-like peptide 1 in Dahl salt-sensitive rats. *J Hypertens* 2003; **21**: 1125–35.

32 Ban K, Noyan-Ashraf MH, Hoefer J, Bolz SS, Drucker DJ, Husain M. Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. *Circulation* 2008; **117**: 2340–50.

33 Noyan-Ashraf MH, Momen MA, Ban K et al. GLP-1R agonist liraglutide activates cytoprotective pathways and improves outcomes after experimental myocardial infarction in mice. *Diabetes* 2009; **58**: 975–83.

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