Annals of Medicine

ISSN: 0785-3890 (Print) 1365-2060 (Online) Journal homepage: informahealthcare.com/journals/iann20

The GLP-1 system as a therapeutic target

Mark C. B. Edwards

Annals of Medicine. 2005; 37: 314–322

Taylor & Francis
Taylor & Francis Group

REVIEW ARTICLE

The GLP-1 system as a therapeutic target

C. MARK B. EDWARDS

Diabeticare, The Hillingdon Hospital, Uxbridge, Middlesex, UK

Abstract
Glucagon-like peptide-1 (GLP-1) was first isolated and sequenced twenty years ago. It has been shown to have many effects in man. GLP-1 stimulates insulin secretion, delays gastric emptying, decreases glucagon levels and reduces appetite, all resulting in a fall in plasma glucose concentrations. More recently, evidence suggests it can stimulate beta cell neogenesis and improve cardiovascular function, and may even be neuroprotective. It is no surprise that this peptide is of increasing interest as a target for the treatment of diabetes. None of the drugs currently available for diabetes are able to achieve targets in all patients and none of them are without side effects. The multiple modes of action of GLP-1 together with its low propensity for hypoglycaemia appear to give it advantages over currently available treatment modalities. In this review I shall examine the data suggesting that medications modelled on the GLP-1 system may provide a new therapeutic option for diabetes in the future.

Key words: *Dipeptidyl peptidase IV, exendin-4, gastric emptying, glucagon, glucagon-like peptide-1, hypoglycaemia, incretin, insulinotropic, LAF237, liraglutide*

Introduction

It is 90 years since Moore et al. first demonstrated that a substance extracted from the mucosa of the gastrointestinal tract could treat diabetes (1). In 1929 Zunz and La Barre named a substance proposed to be released from the gut in response to nutrient ingestion that stimulates insulin secretion 'incretin' (2). It was not until just over 40 years ago that it was demonstrated that oral ingestion of glucose produced higher insulin levels than intravenous infusion of an equivalent dose of glucose (3). It has since been shown that the incretin effect is responsible for at least 50% of meal-induced insulin release in man (4). The incretin effect was defined by Creutzfeldt, who suggested the presence of more than one factor (5). The sequence of glucagon-like peptide-1 [GLP-1] in man was first published in 1983 (6). It is highly conserved in all species implying an important function (7). A number of studies indicated that GLP-1 is the most potent naturally occurring incretin in man (8–9), and has a physiological role in the regulation of postprandial glucose (10).

Actions of GLP-1

Regulation of carbohydrate metabolism

GLP-1 not only stimulates insulin secretion (insulinotropic), but has several other actions which coordinate to reduce plasma glucose. GLP-1 stimulates insulin synthesis (11), and it appears to increase islet cell mass (12), though the mechanism through which this occurs is poorly characterized (13). It also inhibits islet apoptosis (14). Type-2 diabetes is associated with a progressive decline in beta cell function (15), thus, up-regulation of the GLP-1 axis may even slow the progression of diabetes.

GLP-1 reduces glucagon (8,16), which opposes the action of insulin. As the major role of glucagon is in the fasted state (17), and levels in diabetes are high (18), antagonism via the GLP-1 system would be expected to be particularly important in controlling fasting glucose in diabetes. It also reduces glucose by delaying gastric emptying (19–21), and inhibits gastric acid secretion (19). Administration of GLP-1 with a meal in healthy humans causes a reduction in meal-related glucose and insulin excursions, implying that delay in gastric emptying is more

Correspondence: Dr C. Mark B. Edwards, Diabeticare, Hillingdon Hospital, Uxbridge, Middlesex, UB3 3NN, UK. Fax: +44 1895 279521. E-mail: c.m.b.edwards@imperial.ac.uk

ISSN 0785-3890 print/ISSN 1365-2060 online
DOI: 10.1080/07853890510037400

Key messages

- Current therapeutic strategies for type-2 diabetes are inadequate, the disease is rapidly increasing in prevalence and new and better drugs are urgently needed.
- GLP-1 increases plasma insulin, decreases glucagon, delays gastric emptying and reduces appetite whilst causing little or no hypoglycaemia.
- Long-acting GLP-1 analogues resistant to breakdown by dipeptidyl peptidase IV and antagonists of this enzyme are potentially advantageous treatments for diabetes.

important than its insulinotropic effect (20). GLP-1 reduces food intake and act as a satiety factor in rats (22), it can also cause weight loss (23). Further data indicate that it can reduce food intake in man (21,24,25), also likely to help ameliorate diabetes.

A further advantage of GLP-1 as a potential agent to treat diabetes would be if it enhanced glucose disposal independent of insulin. This was suggested (17,26), but most studies have failed to reproduce it (27,28), and the effect remains controversial. GLP-1 is glucose-dependent (29), thus, it reduces glucose to a greater extent in the hyper- than the normoglycaemic state (30). This fact was used to infer that it does not cause fasting hypoglycaemia. In some studies this is the case (31,32), whereas in others hypoglycaemia can occur (30,33–35), however, apparently only in normal volunteers, not people with type 2 diabetes. Overall it seems that therapeutic agents for type-2 diabetes based on GLP-1 will cause little hypoglycaemia, giving it major advantage over insulin or sulphonylureas (36).

Cardiovascular action

GLP-1 has cardiovascular actions though these vary between species. In the rat it increased heart rate and blood pressure (37). In a rat model of hypertension, GLP-1 reduced blood pressure, seemingly via a natriuretic and diuretic action (38). GLP-1 receptor knockout mice have reduced heart rate and increased left ventricular wall thickness (39), consistent with a physiological role in cardiovascular metabolism. In the calf, it is chronotropic without effect on blood pressure (40). In the dog it appeared to enhance recovery from ischaemic myocardial stunning (41).

Cardiovascular effects of GLP-1 in man also vary. Subcutaneous (s/c) injections in healthy volunteers increased blood pressure (34), whereas continuous s/c infusion tended to lower it (33). GLP-1 infusion improved endothelial function in patients with stable coronary artery disease whilst having no effect in normal subjects (42). Intravenous (iv) GLP-1 improved left ventricular function in patients with severe heart failure after large myocardial infarction and successful primary angioplasty (43). Given the frequency of endothelial dysfunction in diabetes, it may have beneficial effects long-term, and may have a short-term role in certain high risk patients.

Action on sodium and water balance

Peripheral administration of GLP-1 inhibited water intake in the rat (44). This effect was abolished by destruction of the arcuate nucleus and circumventricular organs (45). The area postrema forms part of the circumventricular organs and peripheral administration activates this area (46), implying peripheral GLP-1 may be acting centrally. It also acts as a natriuretic and diuretic in the hypertensive rat (38).

As well as a reduction in food intake with GLP-1 administration, a decrease in water intake in healthy volunteers (47) and people with type-2 diabetes (25) has been found. It is natriuretic in healthy human subjects and patients with insulin resistance, suggesting a possible renoprotective effect (48).

Neurological function

GLP-1 may also be neuroprotective. It reduced apoptosis in models of neurodegeneration, (49) and reduced levels of amyloid (50). It improved spatial learning in rats and restored a learning deficit in GLP-1 knockout mice: indeed receptor overexpression improved learning and memory (51). There are no human data reported, however, a beneficial role in Alzheimer's disease has been proposed (50).

Therapeutic principle of GLP-1

GLP-1 is rapidly broken down by dipeptidyl peptidase IV (DPP-IV). The plasma half-life in man is 1–3 minutes (52). Nevertheless, exogenous GLP-1 can reduce plasma glucose in type-2 diabetes (16,53,9). This has been confirmed using s/c injections (54), buccal tablets (55), and s/c infusion (56–59), with additional effect over metformin (56) and glitazones (59). All these studies verified that the GLP-1 system is a target for therapy of type-2 diabetes, alone or in combination providing impetus to the search for a long-acting analogue.

GLP-1 analogues

Exendin-4

Exendin-4, isolated from the salivary gland of the Gila monster, is a long acting GLP-1 receptor agonist (60). The half-life of this peptide in man is about 30 minutes (61), making it a superior prospect to GLP-1 as an anti-diabetic agent. Exendin-4 has a longer lasting and more potent glucose-lowering effect than GLP-1 in diabetic mice (62), rats and monkeys (63). It stimulates beta cell replication and islet neogenesis (64,65) improving glucose tolerance (65), and reduces weight gain in rats (66). Exendin-4 also mimics the neuroprotective effects of GLP-1 (49).

Intravenous infusion of exendin-4 in healthy humans reduced fasting and postprandial glucose, as well as reducing food intake by 19% (61). The dose used in this study may have been close to the maximal tolerated as double this dose caused nausea in some subjects (61). A hyperglycaemic clamp was used to show that iv exendin-4 is a potent insulinotropic agent in type-2 diabetes, with a long duration of action (67). Subcutaneous exendin-4 caused complete abolition of postprandial glycaemia in people with type-2 diabetes, plasma glucose dropping or at least failing to rise after a meal in the active group, associated with a potent slowing of gastric emptying (68). However, a number of subjects suffered nausea and/or vomiting. A reduction of fasting glucose was also noted, though no one was hypoglycaemic (68). Subcutaneous exendin-4 also reduced postprandial hyperglycaemia in type-1 diabetes by 90%, via inhibition of gastric emptying and glucagon suppression (69).

Twice-daily s/c exendin-4 injections for a month in people with type-2 diabetes caused long-term glycaemic control, as measured by glycosylated haemoglobin (HbA1c), to improve by 0.8% (70). There were no significant side-effects, aside from short-lasting nausea at the start of the study with two subjects. There was no hypoglycaemia with the lowest blood glucose being 3.3 mmol/l, but there was no weight loss. Improvement in glycaemic control did not occur for 24 hours, and the authors concluded that exendin-4 in this dose/form would be unlikely to be a treatment for type-2 diabetes (70). Data from Amylin Pharmaceuticals illustrated its effects combined with metformin and/or sulphonylureas (71). Over half the patients were on combined medication. 'Triple' therapy with two or three times a day s/c exendin-4 reduced HbA1c 0.9% in one month, compared with 0.3% in the placebo group. HbA1c indicates average plasma glucose over three months, thus longer trials should have a greater effect. Mild hypoglycaemia did occur, although only in combination with a sulphonylurea: nausea also occurred causing a dropout rate of 3.7% (71). A recent report showed that the nausea caused by exendin-4 can be reduced by dose titration (72).

The first study of exendin-4 for longer than one month has recently been reported by Amylin (73). Patients taking sulphonylureas were randomized to one of two doses of s/c exendin-4 twice a day or placebo for six months. HbA1c decreased by 0.98% with 10 mcg and 0.57% with 5 mcg compared to placebo, the effect was greater in more poorly controlled patients (1.35 and 0.71 respectively if HbA1c $>$ 9%). Study withdrawal was 20%–30% in the treatment arms, and 40% in the placebo arm. The latter was partly due to worse glycaemic control *as per* the protocol, which is likely to have caused a reduction in the measured treatment effect compared with the actual effect. Adverse events causing withdrawal were 3.3% for placebo, 7.2% for low dose and 10.1% for high dose exendin-4. There was a small weight loss of 1.6 kg over the study period in the high dose group; the placebo group lost 0.6 kg; and reported nausea and hypoglycaemia were more common in the treatment groups (73). The improvement in glycaemic control is not unlike that for agents currently used in type-2 diabetes. Data looking at its effects in combination with metformin or with sulphonylureas and metformin have been presented in abstract form.

In 2002 Eli Lilly went into partnership with Amylin to develop exendin-4, also known as exenatide. The peptide currently has more published human data than any other targeting the GLP-1 receptor. A long acting formulation designed for use once a week to once a month is in clinical trials, but no more information is currently available.

Liraglutide

The other GLP-1 agonist with considerable published data is the Novo Nordisk product liraglutide. This is an acylated derivative of GLP-1 that binds to albumin and is resistant to DPP-IV degradation (74). Subcutaneous injection of liraglutide in rats caused weight loss for ten days (75). It attenuated diabetes in two models of pre-diabetes, at least partly via reduction in food intake (76), and induced a short term increase in beta cell mass in normal rats (77). Liraglutide reduced glucose in diabetic *ob*/*ob* and *db*/*db* mice, also increasing beta cell mass in the latter (78). In pigs, it improved glycaemic control chronically, it stimulated insulin, decreased glucagon levels and delayed gastric emptying (79).

The half-life of liraglutide in man is 11–15 hours (80). Injection into people with type-2 diabetes

reduced fasting glucose, and postprandial glucose via stimulation of insulin, reduction of glucagon and delay in gastric emptying (81). Subcutaneous liraglutide once daily for a week in people with type-2 diabetes caused a decrease in average plasma glucose of 2 mmol/L and postprandial glucose after meals of 20 per cent (82). Insulin levels were unchanged, though in the presence of reduced plasma glucose this suggests a relative insulinotropic effect, as has been described with GLP-1 (83). It decreased plasma glucagon and glycogenolysis, improved islet cell function and first phase insulin response, but did not affect gastric emptying (82). There was no hypoglycaemia but nausea and abdominal pain were experienced by some.

An eight-week study of once daily s/c liraglutide reduced HbA1c by 0.8% compared with placebo, with no change in gastric emptying, body weight or energy expenditure (84). Side-effects of medication were minor. These latter two studies (82,84) used a lower dose of liraglutide than that of the previous (81) and it seems likely at higher doses that liraglutide does delay gastric emptying.

Administration of liraglutide for 12 weeks caused a decrease in HbA1c of 0.75% with the highest dose (85). There was a tendency for weight loss, with one dose causing significant weight loss of 1.2 kg compared with placebo. There was minor nausea with increase in dose, but no patients withdrew from the study (85). Acute studies in man using GLP-1 and its agonists have generally shown a decrease in food intake and even weight loss (21,24,25,47,61), however, the chronic effects are much less impressive. Weight loss of 1 kg *versus* placebo was found with exenatide administered for six months (73) and 1.2 kg with one dose of liraglutide for three months (85). I suspect, given the number of studies showing decreased food intake without nausea, that the weight loss is a direct effect, at least at certain doses; however, the metabolic significance may be minor, and dosage is obviously critical. Nevertheless, for prescribers and patients alike the fact that a GLP-1-based drug is likely to cause weight loss, however minimal, compared with the well recognized weight gain of insulin and sulphonylureas and the weight stabilization of metformin (86) will be crucial in the ability of these drugs to compete in the pharmaceutical market.

Other GLP-1 agonists

Various strategies have been used to protect GLP-1 from breakdown by DPP-IV and thus increase the half-life. Acylation or modification of one of the first three amino acids of GLP-1 (7-36 amide) has been most commonly investigated (87). Conjuchem have patented a modified GLP-1, which binds to albumin called CJC-1131, this is currently in phase II clinical trials (88). Human Genome Sciences have a similar albumin-bound peptide, Albugon, which has been shown to have a prolonged action in mice (89).

DPP-IV antagonists

An alternative strategy to target the GLP-1 system, rather than creating DPP-IV resistant analogues, is to inhibit the enzyme itself. DPP-IV knockout mice have reduced glycaemic excursion after a glucose challenge (90) and are protected from both obesity and insulin resistance, implying DPP-IV antagonists are a therapeutic option (91). The enzyme not only inactivates GLP-1, but also glucose-dependent insulinotropic peptide (GIP) (92). Knockout of both the GLP-1 and GIP receptors in a mouse model caused increased glycaemic excursion in response to oral glucose, it also did not respond to DPP-IV inhibition implying that GLP-1 and GIP account for most of the incretin action regulated by DPP-IV (93).

However, a note of caution must be sounded in that DPP-IV, also known as CD26, has many actions. Not only does it have many peptide hormones as substrates, it also stimulates T-cell proliferation as well as inactivating cytokines such as tumour necrosis factor-alpha (TNF-α) (94). Activity of the enzyme alters in a number of disease states, in particular dropping in depression (95). DPP-IV structure is highly conserved in evolution like GLP-1, implying an important physiological role (96) and long-term blockade may have predictable or hitherto unpredictable side-effects (97).

Despite the above, a number of oral DPP-IV inhibitors have been tested in animals, particularly glucose intolerant models, and in man. Several acute studies have been reported: less data are available on the chronic use of DPP-IV antagonists. The Novartis compound DPP728 given long-term to mice improved glucose tolerance with increase in insulin levels (98). The Ferring compound FE 999011 improved glycaemia and delayed the onset of diabetes in Zucker fatty rats (99). Chronic administration of the Probiodrug P32/98 improved glycaemia in Zucker rats (100).

DPP728 has also been shown to be active in people with diet-controlled type-2 diabetes (101). Two or three times a day oral therapy improved average plasma glucose by 1 mmol/L after four weeks compared with placebo, there was no change in body weight. Tablets were taken ten minutes before food, and were generally well tolerated, the

incidence of hypoglycaemia and gastrointestinal side-effects was low, though one patient did develop transient nephrotic syndrome (101).

LAF237 (Vildagliptin)

Novartis have published no further data on DPP728, and have concentrated on the longer acting DPP-IV inhibitor LAF237, also known as vildagliptin. Administration once a day thirty minutes before breakfast for four weeks in people with type-2 diabetes decreased plasma glucose 1 mmol/L. There was little effect on insulin, but glucagon was inhibited. No significant side effects were noted, and there was no change in body weight (102). This group has gone on to perform a longer-term study of vildagliptin in patients treated with metformin (103). After 12 weeks HbA1c dropped 0.7% compared with placebo, after 1 year it was 1.1% in the 58 out of the original 107 patients who continued. Diabetic control at the start of the study was moderate (HbA1c 7.7 or 7.8). There was reduced fasting glucose and glucose area under the curve (AUC) in response to a test meal with vildagliptin, insulin AUC was unchanged indicating a relative insulinotropic effect. No effect on body weight was found and there was a low incidence of hypoglycaemia (103). Three of 42 patients treated with vildagliptin for a year required an increase in their anti-hypertensives; the cause of this is unknown, though this would be consistent with the increase in blood pressure previously found with GLP-1 (34).

Conclusion

Much data has been published on GLP-1 and its agonists in rats and other species. Potentially important theories have been proposed regarding increase in islet cell mass induced by GLP-1, slowing of the progression of diabetes or delay in its onset with GLP-1 and its agonists and even a neuroprotective role. However, these have all just been demonstrated in animals, and are only inferred and have not been reported in man. Nevertheless, several reports have been published in man regarding three first-generation agents targeting the GLP-1 system. Exenatide reduces HbA1c and probably causes some weight loss, it produces little hypoglycaemia, though it has to be injected twice a day, and nausea is likely to limit its usefulness in some. There is a hint of a much longer-acting exendin-like molecule. Less data are available for liraglutide, which seems to have similar efficacy and also may cause some weight loss. It only has to be injected once a day. Vildagliptin is the only oral compound available. It has similar anti-hyperglycaemic effect to the other two but without causing weight loss. We await evidence to suggest whether the less specific DPP-IV antagonism is of long-term advantage or harm.

Currently, the major compounds used in the treatment of type-2 diabetes are metformin, which is not tolerated by many and contra-indicated in many more, sulphonylureas, which cause weight gain and hypoglycaemia, and thiazolidinediones, which appear less efficacious than the others, cause weight gain and have less long-term data. The alternative is insulin; however, the necessary multiple injections are disliked by patients, and weight gain and hypoglycaemia remain problematic. The prevalence of obesity and thence type-2 diabetes is increasing rapidly. There is an urgent need for newer drugs that have the activity profile of agents acting on the GLP-1 system: soon we will see whether any of the agents above fit the bill. Excitingly, we have early evidence suggesting the possibility of advantage in the long term, ranging from cardiovascular to neurological protection, features that would make such drugs even more attractive for the long-term treatment of type-2 diabetes.

References

1. Moore B, Edie ES, Abram JH. On the treatment of diabetes mellitus by the acid extraction of duodenal mucous membranes. Biochem J. 1906;1:28–38.
2. Zunz E, La Barre J. Contributions a l'etude des variations physiologiques de la secretion interne du pancreas: relations entre les secretions externe et interne du pancreas. Arch Int Physiol Biochim Biophys. 1929;31:20–44.
3. Mcintyre N, Holdsworth CD, Turner DS. New interpretation of oral glucose tolerance. Lancet. 1964;41:20–1.
4. Perley MJ, Kipnis DM. Plasma insulin responses to oral and intravenous glucose: studies in normal and diabetic subjects. J Clin Invest. 1967;46:1954–62.
5. Creutzfeldt W. The incretin concept today. Diabetologia. 1979;16:75–85.
6. Bell GI, Sanchez-Pescador R, Laybourn PJ, Najarian RC. Exon duplication and divergence in the human preproglucagon gene. Nature. 1983;304:368–71.
7. Lopez LC, Li WH, Frazier ML, Luo CC, Saunders GF. Evolution of glucagon genes. Mol Biol Evol. 1984;1:335–44.
8. Kreymann B, Williams G, Ghatei MA, Bloom SR. Glucagon-like peptide-1 7-36: a physiological incretin in man. Lancet. 1987;2:1300–4.
9. Nauck MA, Heimesaat MM, Orskov C, Holst JJ, Ebert R, Creutzfeldt W. Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. J Clin Invest. 1993;91:301–7.
10. Edwards CM, Todd JF, Mahmoudi M, Wang Z, Wang RM, Ghatei MA, et al. Glucagon-like peptide 1 has a physiological role in the control of postprandial glucose in humans: studies with the antagonist exendin 9–39. Diabetes. 1999;48:86–93.

11. 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 U S A. 1987;84:3434–8.
12. De Leon DD, Deng S, Madani R, Ahima RS, Drucker DJ, Stoffers DA. Role of endogenous glucagon-like peptide-1 in islet regeneration following partial pancreatectomy. Diabetes. 2003;52:365–71.
13. Wang Q, Brubaker PL. Glucagon-like peptide-1 treatment delays the onset of diabetes in 8 week-old db/db mice. Diabetologia. 2002;45:1263–73.
14. Farilla L, Bulotta A, Hirshberg B, Li Calzi S, Khoury N, Noushmehr H, et al. Glucagon-like peptide 1 inhibits cell apoptosis and improves glucose responsiveness of freshly isolated human islets. Endocrinology. 2003;144:5149–58.
15. UK Prospective Diabetes Study (UKPDS) Group, Glycemic control with diet, sulfonylurea, metformin, or insulin in patients with type 2 diabetes mellitus: progressive requirement for multiple therapies (UKPDS 49). JAMA. 1999;281:2005–12.
16. Gutniak M, Orskov C, Holst JJ, Ahren B, Efendic S. Antidiabetogenic effect of glucagon-like peptide-1 (7-36) amide in normal subjects and patients with diabetes mellitus. N Engl J Med. 1992;326:1316–22.
17. Unger RH. Role of glucagon in the pathogenesis of diabetes: the status of the controversy. Metabolism. 1978;27: 1691–709.
18. Unger RH, Orci L. The essential role of glucagon in the pathogenesis of diabetes mellitus. Lancet. 1975;7897:14–6.
19. Wettergren A, Schjoldager B, Mortensen PE, Myhre J, Christiansen J, Holst JJ. Truncated GLP-1 (proglucagon 78-107-amide) inhibits gastric and pancreatic functions in man. Dig Dis Sci. 1993;38:665–73.
20. Nauck MA, Niedereichholz U, Ettler R, Holst JJ, Orskov C, Ritzel 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.
21. Naslund E, Gutniak M, Skogar S, Rossner S, Hellstrom PM. Glucagon-like peptide 1 increases the period of postprandial satiety and slows gastric emptying in obese men. Am J Clin Nutr. 1998;68:525–30.
22. Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CM, Meeran K, et al. A role for glucagon-like peptide-1 in the central regulation of feeding. Nature. 1996;379:69–72.
23. Meeran K, O'Shea D, Edwards CM, Turton MD, Heath MM, Gunn I, et al. Repeated intracerebroventricular administration of glucagon-like peptide-1-(7-36) amide or exendin-(9-39) alters body weight in the rat. Endocrinology. 1999;140:244–50.
24. 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.
25. Gutzwiller JP, Drewe J, Goke B, Schmidt H, Rohrer B, Lareida J, et al. Glucagon-like peptide-1 promotes satiety and reduces food intake in patients with diabetes mellitus type 2. Am J Physiol. 1999;276:R1541–4.
26. D'Alessio DA, Kahn SE, Leusner CR, Ensinck JW. Glucagon-like peptide 1 enhances glucose tolerance both by stimulation of insulin release and by increasing insulin-independent glucose disposal. J Clin Invest. 1994; 93:2263–6.
27. Orskov L, Holst JJ, Moller J, Orskov C, Moller N, Alberti KG, et al. GLP-1 does not not acutely affect insulin sensitivity in healthy man. Diabetologia. 1996;39:1227–32.
28. Ahrén B, Larsson H, Holst JJ. Effects of glucagon-like peptide-1 on islet function and insulin sensitivity in noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab. 1997;82:473–8.
29. Holz GG 4th, Kuhtreiber WM, Habener JF. Pancreatic beta-cells are rendered glucose-competent by the insulinotropic hormone glucagon-like peptide-1(7-37). Nature. 1993;361:362–5.
30. Ritzel R, Orskov C, Holst JJ, Nauck MA. Pharmacokinetic, insulinotropic, and glucagonostatic properties of GLP-1 [7-36 amide] after subcutaneous injection in healthy volunteers. Dose-response-relationships. Diabetologia. 1995; 38:720–5.
31. Qualmann C, Nauck MA, Holst JJ, Orskov C, Creutzfeldt W. Insulinotropic actions of intravenous glucagon-like peptide-1 (GLP-1) [7-36 amide] in the fasting state in healthy subjects. Acta Diabetol. 1995;32:13–6.
32. Knop FK, Vilsboll T, Larsen S, Madsbad S, Holst JJ, Krarup T. No hypoglycemia after subcutaneous administration of glucagon-like peptide-1 in lean type 2 diabetic patients and in patients with diabetes secondary to chronic pancreatitis. Diabetes Care. 2003;26:2581–7.
33. Toft-Nielsen M, Madsbad S, Holst JJ. Exaggerated secretion of glucagon-like peptide-1 (GLP-1) could cause reactive hypoglycaemia. Diabetologia. 1998;41:1180–6.
34. Edwards CM, Todd JF, Ghatei MA, Bloom SR. Subcutaneous glucagon-like peptide-1 (7-36) amide is insulinotropic and can cause hypoglycaemia in fasted healthy subjects. Clin Sci. 1998;95:719–24.
35. Vilsboll T, Krarup T, Madsbad S, Holst JJ. No reactive hypoglycaemia in Type 2 diabetic patients after subcutaneous administration of GLP-1 and intravenous glucose. Diabet Med. 2001;18:144–9.
36. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet. 1998;352:837–53.
37. Barragan JM, Rodriguez RE, Blazquez E. Changes in arterial blood pressure and heart rate induced by glucagon-like peptide-1-(7-36) amide in rats. Am J Physiol. 1994;266: E459–66.
38. Yu M, Moreno C, Hoagland KM, Dahly A, Ditter K, Mistry M, et al. Antihypertensive effect of glucagon-like peptide 1 in Dahl salt-sensitive rats. J Hypertens. 2003;21:1125–35.
39. Gros R, You X, Baggio LL, Kabir MG, Sadi AM, Mungrue IN, et al. Cardiac function in mice lacking the glucagon-like peptide-1 receptor. Endocrinology. 2003;144: 2242–52.
40. Edwards CM, Edwards AV, Bloom SR. Cardiovascular and pancreatic endocrine responses to glucagon-like peptide-1(7-36) amide in the conscious calf. Exp Physiol. 1997;82:709–16.
41. Nikolaidis LA, Doverspike A, Hentosz T, Zourelias L, Shen YT, Elahi D, et al. Glucagon-like peptide-1 limits myocardial stunning following brief coronary occlusion and reperfusion in conscious canines. J Pharmacol Exp Ther. 2005;312:303–8.
42. Nystrom T, Gutniak MK, Zhang Q, Zhang F, Holst JJ, Ahren B, et al. Effects of glucagon-like peptide-1 on endothelial function in type 2 diabetes patients with stable coronary artery disease. Am J Physiol Endocrinol Metab. 2004;287:E1209–15.
43. Nikolaidis LA, Mankad S, Sokos GG, Miske G, Shah A, Elahi D, et al. Effects of glucagon-like peptide-1 in patients with acute myocardial infarction and left ventricular

dysfunction after successful reperfusion. Circulation. 2004;109:962–5.
44. Tang-Christensen M, Larsen PJ, Goke R, Fink-Jensen A, Jessop DS, Moller M, et al. Central administration of GLP-1-(7-36) amide inhibits food and water intake in rats. Am J Physiol. 1996;271:R848–56.
45. Tang-Christensen M, Vrang N, Larsen PJ. Glucagon-like peptide 1(7-36) amide's central inhibition of feeding and peripheral inhibition of drinking are abolished by neonatal monosodium glutamate treatment. Diabetes. 1998;47: 530–7.
46. Yamamoto H, Kishi T, Lee CE, Choi BJ, Fang H, Hollenberg AN, et al. Glucagon-like peptide-1-responsive catecholamine neurons in the area postrema link peripheral glucagon-like peptide-1 with central autonomic control sites. J Neurosci. 2003;23:2939–46.
47. Gutzwiller JP, Goke B, Drewe J, Hildebrand P, Ketterer S, Handschin D, et al. Glucagon-like peptide-1: a potent regulator of food intake in humans. Gut. 1999;44:81–6.
48. Gutzwiller JP, Tschopp S, Bock A, Zehnder CE, Huber AR, Kreyenbuehl M, et al. Glucagon-like peptide 1 induces natriuresis in healthy subjects and in insulin-resistant obese men. J Clin Endocrinol Metab. 2004;89:3055–61.
49. Perry T, Haughey NJ, Mattson MP, Egan JM, Greig NH. Protection and reversal of excitotoxic neuronal damage by glucagon-like peptide-1 and exendin-4. J Pharmacol Exp Ther. 2002;302:881–8.
50. Perry T, Lahiri DK, Sambamurti K, Chen D, Mattson MP, Egan JM, et al. Glucagon-like peptide-1 decreases endogenous amyloid-beta peptide (Abeta) levels and protects hippocampal neurons from death induced by Abeta and iron. J Neurosci Res. 2003;72:603–12.
51. During MJ, Cao L, Zuzga DS, Francis JS, Fitzsimons HL, Jiao X, et al. Glucagon-like peptide-1 receptor is involved in learning and neuroprotection. Nat Med. 2003;9: 1173–9.
52. Deacon CF, Knudsen LB, Madsen K, Wiberg FC, Jacobsen O, Holst JJ. Dipeptidyl peptidase IV resistant analogues of glucagon-like peptide-1 which have extended metabolic stability and improved biological activity. Diabetologia. 1998;41:271–8.
53. Nauck MA, Kleine N, Orskov C, Holst JJ, Willms B, Creutzfeldt W. Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7-36 amide) in type 2 (non-insulin-dependent) diabetic patients. Diabetologia. 1993;36:741–4.
54. Todd JF, Edwards CM, Ghatei MA, Mather HM, Bloom SR. Subcutaneous glucagon-like peptide-1 improves postprandial glycaemic control over a 3-week period in patients with early type 2 diabetes. Clin Sci. 1998;95:325–9.
55. Gutniak MK, Larsson H, Sanders SW, Juneskans O, Holst JJ, Ahren B. GLP-1 tablet in type 2 diabetes in fasting and postprandial conditions. Diabetes Care. 1997;20: 1874–9.
56. Zander M, Taskiran M, Toft-Nielsen MB, Madsbad S, Holst JJ. Additive glucose-lowering effects of glucagon-like peptide-1 and metformin in type 2 diabetes. Diabetes Care. 2001;24:720–5.
57. 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.
58. Meneilly GS, Greig N, Tildesley H, Habener JF, Egan JM, Elahi D. Effects of 3 months of continuous subcutaneous administration of glucagon-like peptide 1 in elderly patients with type 2 diabetes. Diabetes Care. 2003;26:2835–41.
59. Zander M, Christiansen A, Madsbad S, Holst JJ. Additive effects of glucagon-like peptide 1 and pioglitazone in patients with type 2 diabetes. Diabetes Care. 2004;27: 1910–4.
60. Raufman JP, Singh L, Singh G, Eng J. Truncated glucagon-like peptide-1 interacts with exendin receptors on dispersed acini from guinea pig pancreas. Identification of a mammalian analogue of the reptilian peptide exendin-4. J Biol Chem. 1992;267:21432–7.
61. Edwards CM, Stanley SA, Davis R, Brynes AE, Frost GS, Seal LJ, et al. Exendin-4 reduces fasting and postprandial glucose and decreases energy intake in healthy volunteers. Am J Physiol Endocrinol Metab. 2001;281:E155–61.
62. Greig NH, Holloway HW, De Ore KA, Jani D, Wang Y, Zhou J, et al. Once daily injection of exendin-4 to diabetic mice achieves long-term beneficial effects on blood glucose concentrations. Diabetologia. 1999;42:45–50.
63. Young AA, Gedulin BR, Bhavsar S, Bodkin N, Jodka C, Hansen B, et al. Glucose-lowering and insulin-sensitizing actions of exendin-4: studies in obese diabetic (ob/ob, db/db) mice, diabetic fatty Zucker rats, and diabetic rhesus monkeys (Macaca mulatta). Diabetes. 1999;48: 1026–34.
64. Xu G, Stoffers DA, Habener JF, Bonner-Weir S. Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rats. Diabetes. 1999;48:2270–6.
65. Tourrel C, Bailbe D, Meile MJ, Kergoat M, Portha B. Glucagon-like peptide-1 and exendin-4 stimulate beta-cell neogenesis in streptozotocin-treated newborn rats resulting in persistently improved glucose homeostasis at adult age. Diabetes. 2001;50:1562–70.
66. Szayna M, Doyle ME, Betkey JA, Holloway HW, Spencer RG, Greig NH, et al. Exendin-4 decelerates food intake, weight gain, and fat deposition in Zucker rats. Endocrinology. 2000;141:1936–41.
67. Egan JM, Clocquet AR, Elahi D. The insulinotropic effect of acute exendin-4 administered to humans: comparison of nondiabetic state to type 2 diabetes. J Clin Endocrinol Metab. 2002;87:1282–90.
68. Kolterman OG, Buse JB, Fineman MS, Gaines E, Heintz S, Bicsak TA, et al. Synthetic exendin-4 (exenatide) significantly reduces postprandial and fasting plasma glucose in subjects with type 2 diabetes. J Clin Endocrinol Metab. 2003;88:3082–9.
69. Dupre J, Behme MT, McDonald TJ. Exendin-4 normalized postcibal glycemic excursions in type 1 diabetes. J Clin Endocrinol Metab. 2004;89:3469–73.
70. Egan JM, Meneilly GS, Elahi D. Effects of 1-mo bolus subcutaneous administration of exendin-4 in type 2 diabetes. Am J Physiol Endocrinol Metab. 2003;284:E1072–9.
71. Fineman MS, Bicsak TA, Shen LZ, Taylor K, Gaines E, Varns A, et al. Effect on glycemic control of exenatide (synthetic exendin-4) additive to existing metformin and/or sulfonylurea treatment in patients with type 2 diabetes. Diabetes Care. 2003;26:2370–7.
72. Fineman MS, Shen LZ, Taylor K, Kim DD, Baron AD. Effectiveness of progressive dose-escalation of exenatide (exendin-4) in reducing dose-limiting side effects in subjects with type 2 diabetes. Diabetes Metab Res Rev. 2004;20:411–7.
73. Buse JB, Henry RR, Han J, Kim DD, Fineman MS, Baron AD; Exenatide-113 Clinical Study Group, Effects of exenatide (exendin-4) on glycemic control over 30 weeks in sulfonylurea-treated patients with type 2 diabetes. Diabetes Care. 2004;27:2628–35.

74. Knudsen LB, Nielsen PF, Huusfeldt PO, Johansen NL, Madsen K, Pedersen FZ, et al. Potent derivatives of glucagon-like peptide-1 with pharmacokinetic properties suitable for once daily administration. J Med Chem. 2000;43:1664–9.
75. 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.
76. Sturis J, Gotfredsen CF, Romer J, Rolin B, Ribel U, Brand CL, 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.
77. 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.
78. Rolin B, Larsen MO, Gotfredsen CF, Deacon CF, Carr RD, Wilken M, 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.
79. Ribel U, Larsen MO, Rolin B, Carr RD, Wilken M, Sturis J, 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.
80. Elbrond B, Jakobsen G, Larsen S, Agerso H, Jensen LB, Rolan P, et al. Pharmacokinetics, pharmacodynamics, safety, and tolerability of a single-dose of NN2211, a long-acting glucagon-like peptide 1 derivative, in healthy male subjects. Diabetes Care. 2002;25:1398–404.
81. Juhl CB, Hollingdal M, Sturis J, Jakobsen G, Agerso H, Veldhuis J, et al. Bedtime administration of NN2211, a long-acting GLP-1 derivative, substantially reduces fasting and postprandial glycemia in type 2 diabetes. Diabetes. 2002;51:424–9.
82. Degn KB, Juhl CB, Sturis J, Jakobsen G, Brock B, Chandramouli V, et al. One week's treatment with the long-acting glucagon-like peptide 1 derivative liraglutide (NN2211) markedly improves 24-h glycemia and alpha- and beta-cell function and reduces endogenous glucose release in patients with type 2 diabetes. Diabetes. 2004;53:1187–94.
83. Edwards CM, Bloom SR. The incretins-outdated terminology in man? Diabetologia. 1999;42:1148–9.
84. Harder H, Nielsen L, Tu DT, Astrup A. The effect of liraglutide, a long-acting glucagon-like peptide 1 derivative, on glycemic control, body composition, and 24-h energy expenditure in patients with type 2 diabetes. Diabetes Care. 2004;27:1915–21.
85. Madsbad S, Schmitz O, Ranstam J, Jakobsen G, Matthews DR; 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. 2004;27:1335–42.
86. UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). Lancet. 1998;352:854–65.
87. Green BD, Gault VA, O'harte FP, Flatt PR. Structurally modified analogues of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) as future antidiabetic agents. Curr Pharm Des. 2004; 10:3651–62.
88. Kim JG, Baggio LL, Bridon DP, Castaigne JP, Robitaille MF, Jette L, et al. Development and characterization of a glucagon-like peptide 1-albumin conjugate: the ability to activate the glucagon-like peptide 1 receptor in vivo. Diabetes. 2003;52:751–9.
89. Baggio LL, Huang Q, Brown TJ, Drucker DJ. A recombinant human glucagon-like peptide (GLP)-1-albumin protein (albugon) mimics peptidergic activation of GLP-1 receptor-dependent pathways coupled with satiety, gastrointestinal motility, and glucose homeostasis. Diabetes. 2004; 53:2492–500.
90. Marguet D, Baggio L, Kobayashi T, Bernard AM, Pierres M, Nielsen PF, et al. Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26. Proc Natl Acad Sci U S A. 2000;97:6874–9.
91. Conarello SL, Li Z, Ronan J, Roy RS, Zhu L, Jiang G, et al. Mice lacking dipeptidyl peptidase IV are protected against obesity and insulin resistance. Proc Natl Acad Sci U S A. 2003;100:6825–30.
92. Mentlein R, Gallwitz B, Schmidt WE. Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7-36)amide, peptide histidine methionine and is responsible for their degradation in human serum. Eur J Biochem. 1993;214:829–35.
93. Hansotia T, Baggio LL, Delmeire D, Hinke SA, Yamada Y, Tsukiyama K, et al. Double incretin receptor knockout (DIRKO) mice reveal an essential role for the enteroinsular axis in transducing the glucoregulatory actions of DPP-IV inhibitors. Diabetes. 2004;53:1326–35.
94. Bauvois B, Sanceau J, Wietzerbin J. Human U937 cell surface peptidase activities: characterization and degradative effect on tumor necrosis factor-alpha. Eur J Immunol. 1992;22:923–30.
95. Maes M, De Meester I, Scharpe S, Desnyder R, Ranjan R, Meltzer HY. Alterations in plasma dipeptidyl peptidase IV enzyme activity in depression and schizophrenia: effects of antidepressants and antipsychotic drugs. Acta Psychiatr Scand. 1996;93:1–8.
96. Darmoul D, Lacasa M, Chantret I, Swallow DM, Trugnan G. Isolation of a cDNA probe for the human intestinal dipeptidylpeptidase IV and assignment of the gene locus DPP4 to chromosome 2. Ann Hum Genet. 1990;54:191–7.
97. Hildebrandt M, Reutter W, Arck P, Rose M, Klapp BF. A guardian angel: the involvement of dipeptidyl peptidase IV in psychoneuroendocrine function, nutrition and immune defence. Clin Sci. 2000;99:93–104.
98. Reimer MK, Holst JJ, Ahren B. Long-term inhibition of dipeptidyl peptidase IV improves glucose tolerance and preserves islet function in mice. Eur J Endocrinol. 2002;146:717–27.
99. Sudre B, Broqua P, White RB, Ashworth D, Evans DM, Haigh R, et al. Chronic inhibition of circulating dipeptidyl peptidase IV by FE 999011 delays the occurrence of diabetes in male zucker diabetic fatty rats. Diabetes. 2002;51:1461–9.
100. Pospisilik JA, Stafford SG, Demuth HU, Brownsey R, Parkhouse W, Finegood DT, et al. Long-term treatment with the dipeptidyl peptidase IV inhibitor P32/98 causes sustained improvements in glucose tolerance, insulin sensitivity, hyperinsulinemia, and beta-cell glucose responsiveness in VDF (fa/fa) Zucker rats. Diabetes. 2002;51: 943–50.
101. Ahren B, Simonsson E, Larsson H, Landin-Olsson M, Torgeirsson H, Jansson PA, et al. Inhibition of dipeptidyl

peptidase IV improves metabolic control over a 4-week study period in type 2 diabetes. Diabetes Care. 2002; 25:869–75.

102. Ahren B, Landin-Olsson M, Jansson PA, Svensson M, Holmes D, Schweizer A. Inhibition of dipeptidyl peptidase-4 reduces glycemia, sustains insulin levels, and reduces glucagon levels in type 2 diabetes. J Clin Endocrinol Metab. 2004;89:2078–84.
103. Ahren B, Gomis R, Standl E, Mills D, Schweizer A. Twelve- and 52-week efficacy of the dipeptidyl peptidase IV inhibitor LAF237 in metformin-treated patients with type 2 diabetes. Diabetes Care. 2004;27:2874–80.