

GLP-1: target for a new class of antidiabetic agents?

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In 1998 a large highly powered trial, the UK Prospective Diabetes Study (UKPDS), demonstrated that in type 2 diabetes several clinical endpoints were improved by better diabetic control. A 0.9% decrease in glycosylated haemoglobin (HbA1c) over ten years was associated with a 25% reduction in microvascular complications ($P=0.0099$) and a less impressive 16% reduction in myocardial infarction ($P=0.052$).¹ With this evidence base, the pressure is on for physicians to improve the HbA1c of people with diabetes.

CURRENT ANTIDIABETIC DRUGS

Therapies currently available for type 2 diabetes are limited, and all have drawbacks. Metformin, which reduced mortality and morbidity in obese people with type 2 diabetes in the UKPDS, is widely used as therapy for such patients.² Contraindications are renal failure, heart failure and liver dysfunction but these are relative: in one study a quarter of patients treated with it had contraindications,³ and many clinicians use metformin despite moderate renal dysfunction (e.g. creatinine less than $150\text{ }\mu\text{mol/L}$), chronic stable heart failure or mild liver dysfunction when transaminases are less than three times normal. A rare side-effect is lactic acidosis, probably avoidable if the drug is not used in severe renal or liver failure.⁴ In addition, many patients cannot tolerate the gastrointestinal side-effects.⁵

The other compounds commonly used to treat type 2 diabetes are sulphonylureas. These drugs have likewise been shown to improve morbidity (at least microvascular).¹ However, sulphonylureas carry a substantial risk of hypoglycaemia,⁶ particularly in elderly patients and those with poor renal function. The rate of major hypoglycaemic events in the UKPDS was 1–1.4% per year. In addition, sulphonylureas caused weight gain of 1.7–2.6 kg.¹ Metformin and sulphonylureas are commonly prescribed in combination; however, in the UKPDS, the group in whom metformin was added to a sulphonylurea had a 96% higher rate of diabetes-related death than those treated with sulphonylureas alone.² Although the metformin-sulphonylurea group was small and this statistical analysis was not a primary endpoint of the study, concern remains regarding the combination.⁷ Two new sulphonylurea-like drugs,

nateglinide and repaglinide, bind to the sulphonylurea receptor and stimulate insulin secretion. Their action is short-lasting and hypoglycaemic episodes are less troublesome than with established sulphonylureas; however, their usefulness alone appears limited to early type 2 diabetes and, even then, they may be less effective than established agents in reducing HbA1c.^{8,9}

The alpha-glucosidase inhibitor acarbose delays intestinal carbohydrate absorption. It appears less efficacious than other antidiabetic drugs¹⁰ and has not proved as successful—not least because of its gastrointestinal side-effects. In contrast, the thiazolidinediones are increasingly prescribed. Rosiglitazone and pioglitazone are peroxisome-proliferator-activated receptor gamma ($\text{PPAR}\gamma$) agonists which alter transcription of several genes involved in carbohydrate and lipid metabolism. These agents decrease insulin resistance¹¹ and seem to be as potent as sulphonylureas or metformin.^{12,13} Unfortunately, thiazolidinediones induce weight gain and can cause fluid retention and are thus contraindicated in heart failure. In recent NICE guidelines thiazolidinediones are recommended only in combination with metformin or sulphonylureas, in patients who either cannot tolerate a combination of the latter two drugs through side-effects or have a contraindication to one of them. In reality, clinicians are starting to use thiazolidinediones outside the NICE guidelines and even beyond the terms of the UK drug licence, particularly as triple therapy with sulphonylureas and metformin. There is very little published information on the safety or efficacy of triple therapy; glycaemic control does seem to improve, though at the expense of more hypoglycaemic events, weight gain and oedema.¹⁴

GLP-1

All the current treatments for type 2 diabetes have important limitations, so the search is on for alternatives. Glucagon-like peptide-1 (GLP-1) analogues seem an attractive possibility.

With oral ingestion of glucose, plasma concentrations of insulin are about twice those induced by intravenous infusion of an equivalent dose of glucose.¹⁵ GLP-1 and GIP (glucose-dependent insulintropic peptide) are responsible for most of the differences between these two values, known as the incretin effect.¹⁶ GLP-1 has a physiological role in the incretin effect in some species though not

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all.¹⁷⁻¹⁹ When infused in people with type 2 diabetes, GIP appears ineffective.²⁰ By contrast, GLP-1 decreases glucose levels,^{20,21} stimulates insulin secretion, decreases glucagon, delays gastric emptying, reduces food intake, stimulates beta-cell neogenesis, may enhance insulin sensitivity,²² and may inhibit beta-cell apoptosis.²³ Its effect is glucose-dependent, lessening though probably not removing the risk of hypoglycaemia.^{24,25} Thus, GLP-1 has several potential advantages over current treatments for type 2 diabetes. 'Proof of principle' for GLP-1 as a therapeutic agent was demonstrated by regular subcutaneous injections²⁶ and by intravenous²⁷ or subcutaneous infusion.^{28,29} The circulating half-life of GLP-1 is about one minute,³⁰ making it an unlikely diabetic agent, but several strategies have been explored to utilize the principle.

GLP-1 is broken down by the enzyme dipeptidyl peptidase IV (DPP IV).^{31,32} Mice lacking this enzyme (DPP IV knockout) show better glucose tolerance, higher GLP-1 levels and greater insulin sensitivity than their non-knockout equivalents,³³ and less obesity and insulin resistance when fed a high fat diet.³⁴ Various DPP IV antagonists and DPP IV resistant analogues of GLP-1 are under investigation.

DPP IV ANTAGONISTS

P32/98, NVP-DPP728 and FE 999011 are DPP IV antagonists. Treatment of Zucker fatty rats (a model of type 2 diabetes) with P32/98 for three months caused sustained improvement in glucose tolerance,³⁵ and mice fed a standard or high-fat diet had better glycaemic control after eight weeks of NVP-DPP728.³⁶ P32/98 stimulated islet neogenesis and beta-cell survival in rats with streptozotocin-induced diabetes, suggesting possible usefulness in type 1 or late type 2 diabetes.³⁷ Administration of FE 999011 to Zucker rats for seven days delayed the onset of diabetes.³⁸

Published work with DPP IV in man is limited. Twice or three times daily oral treatment with NVP-DPP728 for four weeks reduced HbA1c by 0.5%.³⁹ Fasting, postprandial and mean 24-hour glucoses were all reduced, but body weight was unchanged. The medication was generally well tolerated in this patient group, although one out of a group of sixty-five developed transient nephrotic syndrome and was withdrawn from the study.³⁹ Pharmacokinetic assessment of NVP-DPP728 and its daughter compound NVP-LAF237 in monkeys indicates that NVP-LAF237 is suitable for once daily administration and this seems a better therapeutic option, though both products are currently in phase II clinical testing.⁴⁰

DPP IV is not specific to GLP-1 and breaks down several other peptides including neuropeptide Y, peptide YY and GIP as well as chemokines such as macrophage-derived chemokine and eotaxin.⁴¹ Whether increases in the

half-lives of some or all of these compounds will cause side-effects awaits further evaluation.

GLP-1 ANALOGUES

Long-acting GLP-1 receptor agonists such as exendin-4 and liraglutide (NN2211) are also under therapeutic investigation. Exendin-4, isolated from the salivary gland of the Gila monster (*Heloderma suspectum* [Figure 1]), has 53% sequence homology to GLP-1 and is a high-affinity GLP-1 receptor agonist.⁴² Exendin-4 has a longer duration of action than GLP-1: it improved glycaemic control in diabetic mice, rats and baboons and decreased food intake and body weight in Zucker rats.^{43,44} Exendin-4 stimulated beta-cell replication and neogenesis, improving glucose tolerance,⁴⁵ and stimulated non-insulin-secreting pancreatic cells into producing insulin.⁴⁶ The beta-cell neogenesis may occur via increased expression of the homeodomain protein IDX-1,⁴⁷ the lack of which results in failure of pancreas development.⁴⁸ Interestingly, transgenic mice processing excess exendin-4 exhibited improved glucose tolerance and ate less food in the short term but had normal beta-cell mass and islet histology.⁴⁹ Injection of exogenous exendin-4 to streptozotocin treated newborn rats increased beta-cell mass though glucose-stimulated insulin secretion was unaltered.⁵⁰ Exendin-4 increased beta-cell mass and delayed the onset of diabetes when administered in the prediabetic state to two rodent models of diabetes.^{51,52}

Intravenous infusion of exendin-4 in healthy volunteers was well tolerated at one dose but doubling of the dose caused postprandial nausea in some and trebling caused vomiting in most.⁵³ The half-life of intravenous exendin-4



Figure 1 The Gila monster (*Heloderma suspectum*) (courtesy of Dr Mark Seward, www.drseward.com)

was about 30 minutes. It decreased fasting and postprandial glucose and reduced food intake by 19%.⁵³ Exendin-4 seems not to affect insulin sensitivity in healthy volunteers.⁵⁴

More data are available for exendin-4 (exenatide is the synthetic peptide) than for the DPP IV antagonists. Exenatide is insulinotropic in healthy volunteers and people with type 2 diabetes.⁵⁵ Subcutaneous injection of exenatide prevented any postprandial rise in glucose for 300 minutes in people with diabetes whether on diet, oral antidiabetic agents or insulin.⁵⁶ This effect seemed partly due to delayed gastric emptying and glucagon suppression. Exenatide decreased fasting glucose with a maximum effect 3–4 hours after subcutaneous injection, seemingly via insulin stimulation. Though no individuals withdrew from these studies there were slightly more gastrointestinal side-effects in the treatment groups.⁵⁶

Subcutaneous injection of exenatide, in patients on no other antidiabetic therapy⁵⁷ or as an additive treatment to metformin or sulphonylureas,⁵⁸ caused a drop in HbA1c of 0.8% and 0.6%, respectively. However, there was no control group in the first study⁵⁷ and neither study showed a change in body weight. Serious side-effects were rare; reported nausea declined over time and hypoglycaemia occurred only in patients also taking sulphonylureas. There was no difference between twice daily and three times daily dosing,⁵⁸ but once daily was insufficient.⁵⁷ Exenatide therapy is likely to require twice daily subcutaneous injections, although a long-acting preparation is under investigation.⁵⁹

Liraglutide is an acylated derivative of GLP-1 with an aminoacid substitution at position 34 protecting it from DPP IV degradation and increasing the half-life to about 14 hours in pigs.⁶⁰ Twice daily subcutaneous injections of liraglutide for ten days reduced body weight in normal and obese rats,⁶¹ and a similar protocol in *ob/ob* and *db/db* diabetic mice for two weeks improved glycaemic control and increased beta-cell mass (though only significantly in the *db/db* mice).⁶² Twice daily injections of liraglutide for six weeks caused weight loss in normal rats.⁶³

Subcutaneous liraglutide has a half life of 11–15 hours in healthy volunteers.⁶⁴ Subcutaneous injection of liraglutide at night to people with type 2 diabetes decreased fasting glucose the next morning as well as postprandial glucose 12.5 hours later, seemingly at least partly via delayed gastric emptying, glucagon suppression and stimulation of insulin.⁶⁵ The two patients with the highest concentrations of liraglutide developed nausea and one was unable to eat the meal. Liraglutide was more potent during hyperglycaemia when administered before a graded glucose infusion in people with type 2 diabetes.⁶⁶ No long-term studies are published to date. Exenatide and liraglutide are both the subjects of continued clinical trials.

Another method for protection of GLP-1 from degradation by DPP IV is via drug affinity complex technology. A preparation in which CJC-1131 binds GLP-1 to albumin *in vivo* gives better glycaemic control in mice. No human data are available, though the product is in phase II trials.⁶⁷ DPP IV inactivates GLP-1 by removal of the N-terminal dipeptide His(7)-Ala(8).³² Several GLP-1 analogues with longer half-lives have been assessed, particularly those with substitutions or insertions of aminoacids at positions 7, 8 or 9.^{68–72} To date there are no publications of the effects of these compounds in people with type 2 diabetes.

CONCLUSIONS

The range of drugs against diabetes is growing but is still inadequate. DPP IV antagonists and GLP-1 analogues have advantages over current therapies, particularly in terms of hypoglycaemia risk and potential weight loss. NVP-LAF237 appears the most advantageous DPP IV antagonist. It is an oral agent, seemingly without side-effects. However, the likely increase in other products of DPP IV inhibition, together with multiple daily dosing, reduces its potential impact. More clinical data are available for the GLP-1 analogue exenatide. Exenatide causes weight loss and may result in beta-cell restoration, but it seems to produce nausea and has to be injected.

Therapy for diabetes will probably not alter radically in the next few years unless long-term data demonstrate other advantages over metformin and insulin. However, since the number of people with diabetes is increasing rapidly, agents modulating GLP-1 are likely to be licensed, with second or third generation molecules possibly playing a major role in combating the world-wide burden of diabetes in the 21st century.

REFERENCES

- 1 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
- 2 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
- 3 Emslie-Smith AM, Boyle DI, Evans JM, Sullivan F, Morris AD, for the DARTS/MEMO collaboration. Contraindications to metformin therapy in patients with type 2 diabetes—a population-based study of adherence to prescribing guidelines. *Diabetic Med* 2001;**18**:483–8
- 4 Edwards CM, Barton MA, Snook J, David M, Mak VH, Chowdhury TA. Metformin-associated lactic acidosis in a patient with liver disease. *Quart J Med* 2003;**96**:315–16
- 5 Bailey CJ, Turner RC. Metformin. *N Engl J Med* 1996;**334**:574–9
- 6 Lebovitz HE, Melander A. Sulphonylureas: basic aspects and clinical uses. In: Alberti KGMM, DeFronzo RA, Keen H, Zimmet P, eds. *International Textbook of Diabetes Mellitus*, 1st edn. Oxford: Alden Press, 1992:745–72

- 7 Nathan DM. Some answers, more controversy, from UKPDS. United Kingdom Prospective Diabetes Study. *Lancet* 1998;**352**:832–3
- 8 Dornhorst A. Insulinotropic meglitinide analogues. *Lancet* 2001;**358**:1709–16
- 9 Saloranta C, Hershon K, Ball M, Dickinson S, Holmes D. Efficacy and safety of nateglinide in type 2 diabetic patients with modest fasting hyperglycemia. *J Clin Endocrinol Metab* 2002;**87**:4171–6
- 10 Coniff RF, Shapiro JA, Seaton TB, Bray GA. Multicenter, placebo-controlled trial comparing acarbose (BAY g 5421) with placebo, tolbutamide, and tolbutamide-plus-acarbose in non-insulin-dependent diabetes mellitus. *Am J Med* 1995;**98**:443–51
- 11 Nolan JJ, Ludvik B, Beerdsen P, Joyce M, Olefsky J. Improvement in glucose tolerance and insulin resistance in obese subjects treated with troglitazone. *N Engl J Med* 1994;**331**:1188–93
- 12 Lebovitz HE, Dole JF, Patwardhan R, Rappaport EB, Freed MI, Rosiglitazone Clinical Trials Study Group. Rosiglitazone monotherapy is effective in patients with type 2 diabetes. *J Clin Endocrinol Metab* 2001;**86**:280–8
- 13 Aronoff S, Rosenblatt S, Braithwaite S, Egan JW, Mathisen AL, Schneider RL. Pioglitazone hydrochloride monotherapy improves glycemic control in the treatment of patients with type 2 diabetes: a 6-month randomized placebo-controlled dose-response study. The Pioglitazone 001 Study Group. *Diabetes Care* 2000;**23**:1605–11
- 14 Yale JF, Valiquett TR, Ghazzi MN, Owens-Grillo JK, Whitcomb RW, Foyt HL. The effect of a thiazolidinedione drug, troglitazone, on glycemia in patients with type 2 diabetes mellitus poorly controlled with sulfonylurea and metformin. A multicenter, randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 2001;**134**:737–45
- 15 Creutzfeldt W, Ebert R. New developments in the incretin concept. *Diabetologia* 1985;**28**:565–73
- 16 Nauck MA, Bartels E, Orskov C, Ebert R, Creutzfeldt W. Additive insulinotropic effects of exogenous synthetic human gastric inhibitory polypeptide and glucagon-like peptide-1-(7-36) amide infused at near-physiological insulinotropic hormone and glucose concentrations. *J Clin Endocrinol Metab* 1993;**76**:912–17
- 17 Scrocchi LA, Brown TJ, McClusky N, *et al.* Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene. *Nat Med* 1996;**2**:1254–8
- 18 Edwards CM, Todd JF, Mahmoudi M, *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
- 19 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
- 20 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
- 21 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
- 22 Drucker DJ. Minireview: the glucagon-like peptides. *Endocrinology* 2001;**142**:521–7
- 23 Farilla L, Bulotta A, Hirshberg B, *et al.* Glucagon-like peptide 1 inhibits cell apoptosis and improves glucose responsiveness of freshly isolated human islets. *Endocrinology* 2003;**144**:5149–58
- 24 Nathan DM, Schreiber E, Fogel H, Mojsov S, Habener JF. Insulinotropic action of glucagonlike peptide-I-(7-37) in diabetic and nondiabetic subjects. *Diabetes Care* 1992;**15**:270–6
- 25 Edwards CM, Todd JF, Gbatei MA, Bloom SR. Subcutaneous glucagon-like peptide-1 (7-36) amide is insulinotropic and can cause hypoglycaemia in fasted healthy subjects. *Clin Sci (Lond)* 1998;**95**:719–24
- 26 Todd JF, Edwards CM, Gbatei 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 (Lond)* 1998;**95**:325–9
- 27 Rachman J, Barrow BA, Levy JC, Turner RC. Near-normalisation of diurnal glucose concentrations by continuous administration of glucagon-like peptide-1 (GLP-1) in subjects with NIDDM. *Diabetologia* 1997;**40**:205–11
- 28 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
- 29 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
- 30 Deacon CF, Pridal L, Klarskov L, Olesen M, Holst JJ. Glucagon-like peptide 1 undergoes differential tissue-specific metabolism in the anesthetized pig. *Am J Physiol Endocrinol Metab* 1996;**271**:E458–64
- 31 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
- 32 Deacon CF, Johnsen AH, Holst JJ. Degradation of glucagon-like peptide-1 by human plasma *in vitro* yields an N-terminally truncated peptide that is a major endogenous metabolite *in vivo*. *J Clin Endocrinol Metab* 1995;**80**:952–7
- 33 Marguet D, Baggio L, Kobayashi T, *et al.* Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26. *Proc Natl Acad Sci USA* 2000;**97**:6874–9
- 34 Conarello SL, Li Z, Ronan J, *et al.* Mice lacking dipeptidyl peptidase IV are protected against obesity and insulin resistance. *Proc Natl Acad Sci USA* 2003;**100**:6825–30
- 35 Pospisilik JA, Stafford SG, Demuth HU, *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
- 36 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
- 37 Pospisilik JA, Martin J, Doty T, *et al.* Dipeptidyl peptidase IV inhibitor treatment stimulates beta-cell survival and islet neogenesis in streptozotocin-induced diabetic rats. *Diabetes* 2003;**52**:741–50
- 38 Sudre B, Broqua P, White RB, *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
- 39 Ahren B, Simonsson E, Larsson II, *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
- 40 Villhauer EB, Brinkman JA, Naderi GB, *et al.* J-[3-hydroxy-1-adamantyl]amino]acetyl]-2-cyano-(S)-pyrrolidine: a potent, selective, and orally bioavailable dipeptidyl peptidase IV inhibitor with antihyperglycemic properties. *J Med Chem* 2003;**46**:2774–89
- 41 Mentlein R. Dipeptidyl-peptidase IV (CD26)—role in the inactivation of regulatory peptides. *Regul Pept* 1999;**85**:9–24
- 42 Thorens B, Porret A, Buhler L, Deng SP, Morel P, Widmann C. Cloning and functional expression of the human islet GLP-1 receptor. Demonstration that exendin-4 is an agonist and exendin-(9-39) an antagonist of the receptor. *Diabetes* 1993;**42**:1678–82
- 43 Young AA, Gedulin BR, Bhavsar S, *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

- 44 Szayna M, Doyle ME, Betkey JA, *et al.* Exendin-4 decelerates food intake, weight gain, and fat deposition in Zucker rats. *Endocrinology* 2000;**141**:1936–41
- 45 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
- 46 Zhou J, Wang X, Pineyro MA, Egan JM. Glucagon-like peptide 1 and exendin-4 convert pancreatic AR42J cells into glucagon- and insulin-producing cells. *Diabetes* 1999;**48**:2358–66
- 47 Stoffers DA, Kieffer TJ, Hussain MA, *et al.* Insulinotropic glucagon-like peptide 1 agonists stimulate expression of homeodomain protein IDX-1 and increase islet size in mouse pancreas. *Diabetes* 2000;**49**:741–8
- 48 Jonsson J, Carlsson L, Edlund T, Edlund H. Insulin-promoter-factor 1 is required for pancreas development in mice. *Nature* 1994;**371**:606–9
- 49 Baggio L, Adatia F, Bock T, Brubaker PL, Drucker DJ. Sustained expression of exendin-4 does not perturb glucose homeostasis, beta-cell mass, or food intake in metallothionein–preproexendin transgenic mice. *J Biol Chem* 2000;**275**:34471–7
- 50 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
- 51 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
- 52 Stoffers DA, Desai BM, DeLeon DD, Simmons RA. Neonatal exendin-4 prevents the development of diabetes in the intrauterine growth retarded rat. *Diabetes* 2003;**52**:734–40
- 53 Edwards CM, Stanley SA, Davis R, *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
- 54 Vella A, Shah P, Reed AS, Adkins AS, Basu R, Rizza RA. Lack of effect of exendin-4 and glucagon-like peptide-1-(7,36)-amide on insulin action in non-diabetic humans. *Diabetologia* 2002;**45**:1410–15
- 55 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
- 56 Kolterman OG, Buse JB, Fineman MS, *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
- 57 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
- 58 Fineman MS, Bicsak TA, Shen LZ, *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
- 59 Gedulin B, Smith P, Prickett K, *et al.* Dose-response for 2-day glycemic improvement following a single injection of long-acting-release exenatide (synthetic exendin-4) in diabetic fatty Zucker (ZDF) rats. *Diabetes* 2003;**52**(suppl. 1):A109
- 60 Knudsen LB, Nielsen PF, Huusfeldt P, *et al.* Potent derivatives of glucagon-like peptide-1 with pharmacokinetic properties suitable for once daily administration. *J Med Chem* 2000;**43**:1664–9
- 61 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
- 62 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
- 63 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
- 64 Elbrond B, Jakobsen G, Larsen S, *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
- 65 Juhl CB, Hollingdal M, Sturis 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
- 66 Chang AM, Jakobsen G, Sturis J, *et al.* The GLP-1 derivative NN2211 restores beta-cell sensitivity to glucose in type 2 diabetic patients after a single dose. *Diabetes* 2003;**52**:1786–91
- 67 Kim JG, Baggio LL, Bridon DP, *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
- 68 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
- 69 Burcelin R, Dolci W, Thorens B. Long-lasting antidiabetic effect of a dipeptidyl peptidase IV-resistant analog of glucagon-like peptide-1. *Metabolism* 1999;**48**:252–8
- 70 Doyle ME, Greig NH, Holloway HW, Betkey JA, Bernier M, Egan JM. Insertion of an N-terminal 6-aminohexanoic acid after the 7 amino acid position of glucagon-like peptide-1 produces a long-acting hypoglycemic agent. *Endocrinology* 2001;**142**:4462–8
- 71 Naslund E, Skogar S, Efendic S, Hellstrom PM. Glucagon-like peptide-1 analogue LY315902: effect on intestinal motility and release of insulin and somatostatin. *Regul Pept* 2002;**106**:89–95
- 72 Green BD, Gault VA, Mooney MH, *et al.* Novel dipeptidyl peptidase IV resistant analogues of glucagon-like peptide-1(7-36)amide have preserved biological activities *in vitro* conferring improved glucose-lowering action *in vivo*. *J Mol Endocrinol* 2003;**31**:529–40