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Glucagon-like peptide 1 and inhibitors of dipeptidyl peptidase IV in the treatment of type 2 diabetes mellitus

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Proof-of-concept for the efficacy of a glucagon-like peptide 1 (GLP-1)-based therapy of patients with type 2 diabetes was provided in 2002 by means of prolonged continuous subcutaneous infusion of native GLP-1. Since then, several long-acting analogues of GLP-1, as well as inhibitors of dipeptidyl peptidase IV, the enzyme that rapidly inactivates endogenous GLP-1, have demonstrated efficacy in long term clinical trials.

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Abbreviations

ADA	American Diabetes Association
DPP-IV	dipeptidyl peptidase IV
GLP-1	glucagon-like peptide 1
HbA1c	glycated haemoglobin
SU	sulfonylurea
T2DM	type 2 diabetes mellitus

Introduction

Glucagon-like peptide 1 (GLP-1) is a 30 amino acid peptide secreted by intestinal L-cells in response to meal ingestion. It functions as one of the incretin hormones; that is, the gut hormones that enhance nutrient-stimulated insulin secretion more than the nutrients themselves, if given intravenously [1]. In patients with type 2 diabetes mellitus (T2DM), the incretin effect is severely impaired or absent [2], and it is probable that this deficiency contributes to the deficient insulin secretion that characterizes T2DM [3]. The causes of the deficient incretin effect in patients with T2DM have been analysed [3] and seem to comprise an impaired secretion of GLP-1, an impaired sensitivity of the β-cell to the actions of GLP-1 (whereas the efficacy is at least partially preserved) [4], and an abolished effect of glucose-dependent insulinotropic polypeptide on second-phase insulin secretion [5]. In agreement with this, intravenous infusions of GLP-1 in near physiological amounts have been shown to almost completely normalize glucose metabolism in patients with T2DM [6,7]. Because of this, there is currently great interest in trying to develop GLP-1 as a new therapeutic agent for T2DM [8]. However, GLP-1 has many more actions than merely stimulating insulin secretion, and all of these seem to be expedient in the context of diabetes therapy.

Actions of GLP-1

GLP-1 potently stimulates insulin secretion in a strictly glucose-dependent manner. Binding of GLP-1 to the GLP-1 receptor of β-cells causes activation — via a stimulatory G protein — of adenylate cyclase, resulting in the formation of cAMP. Subsequent activation of protein kinase A and the cAMP-regulated guanine nucleotide exchange factor II (also known as Epac2) leads to a plethora of events including altered ion channel activity, intracellular calcium handling and enhanced exocytosis of insulin-containing granules [1]. The clinical implication of the dependence on blood glucose concentrations at or above normal fasting glucose levels is that GLP-1 is incapable of causing profound hypoglycaemia (except perhaps in the presence of sulfonylurea (SU) drugs; see below).

GLP-1 stimulates all steps of insulin biosynthesis, as well as insulin gene transcription [9], thereby providing continued and augmented supplies of insulin for secretion. Activation of PDX-1, a key regulator of islet growth and insulin gene transcription, might be involved [10]. In addition, GLP-1 upregulates genes for the cellular machinery involved in insulin secretion, such as glucokinase and glucose transporter-2 genes [10].

GLP-1 has been shown to have trophic effects on β-cells [11]: not only does it stimulate β-cell proliferation [12,13] but it also enhances the differentiation of new β-cells from progenitor cells in the pancreatic duct epithelium [14]. Proliferation was also induced in aging glucose-intolerant rats, with a resulting improvement in glucose tolerance [15]. Most recently, GLP-1 has been shown to inhibit apoptosis of β-cells, including human β-cells [16••]. Because the normal number of β-cells is maintained in a balance between apoptosis and proliferation, this observation is of considerable interest, and also raises the possibility that GLP-1 could be useful in conditions with increased β-cell apoptosis (e.g. when cells are exposed to the toxic effects of hyperglycaemia and hyperlipidaemia).

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GLP-1 also strongly inhibits glucagon secretion. In patients with T2DM, there is fasting hyperglucagonemia as well as exaggerated glucagon responses to meal ingestion [17]; therefore, it is likely that the hyperglucagonemia contributes to the hyperglycemia of the patients. This effect could be as important as the insulinotropic effects.

Further important effects of GLP-1 include inhibition of gastrointestinal secretion and motility, notably gastric emptying [18,19]. This effect is desirable in patients with diabetes because the slower gastric emptying rate reduces postprandial glucose excursions; the clinical importance of this is evident from the use of another potent gastric inhibitor, amylin, for diabetes treatment [20].

GLP-1 also inhibits appetite and food intake. This has been demonstrated in both normal subjects, obese subjects and subjects with T2DM [21], and it is likely that GLP-1 is one of the physiological regulators of appetite and food intake.

GLP-1 has cardiovascular actions, as it has been known for some time that there are GLP-1 receptors in the heart [22]. A physiological function for these receptors was indicated in recent studies in mice lacking the GLP-1 receptor, which exhibit impaired left ventricular contractility and diastolic functions, as well as impaired responses to exogenous epinephrine [23•]. Recent studies in rats showed that GLP-1 protects the ischaemic and reperfused myocardium in rats by mechanisms independent of insulin [24••]. These findings could have important clinical implications. Thus, Nikolaidis *et al.* [25] studied patients treated with angioplasty after acute myocardial infarction, with postoperative left ventricular ejection fractions as low as 29%. In these patients, GLP-1 administration significantly improved the ejection fraction to 39% and improved both global and regional wall motion indices. Cerebral GLP-1 receptor stimulation increases blood pressure and heart rate and activates autonomic regulatory neurons in rats, leading to downstream activation of cardiovascular responses [26]. Furthermore, it has been suggested that catecholaminergic neurons in the area postrema expressing the GLP-1 receptor may link peripheral GLP-1 and central autonomic control sites that mediate the diverse neuroendocrine and autonomic actions of peripheral GLP-1 [27]. It should be noted, however, that peripheral administration of GLP-1 in humans is not associated with changes in blood pressure or heart rate [28].

Recent studies showed that intracerebroventricular administration of GLP-1 was associated with improved learning in rats and neuroprotective effects [29,30]. GLP-1 has been proposed as a new therapeutic agent for neurodegenerative diseases, including Alzheimer's disease [31•].

Actions of native GLP-1 in type 2 diabetes

These actions render GLP-1 highly attractive as a therapeutic agent, but an extremely rapid enzymatic degradation of the molecule makes it unsuitable for injection therapy. This metabolism, which is attributable to the actions of the ubiquitous enzyme dipeptidyl peptidase IV (DPP-IV), results in a half-life for GLP-1 of only about two minutes [32]; furthermore, the actions on metabolism of single subcutaneous injections are short-lived. However, continuous subcutaneous infusion using insulin pumps was employed in a study where the hormone was given for six weeks to probe its effects in patients with T2DM [28]. Patients were evaluated before, after one week and after six weeks of treatment. No changes were observed in the saline-treated group, whereas in the GLP-1 group fasting and average plasma glucose concentrations were lowered by approximately 5 mmol/l; glycated haemoglobin (HbA1c; a long-term [months] measure of mean plasma glucose concentrations) decreased by 1.2%; free fatty acids were significantly lowered; and the patients had a gradual weight loss of approximately 2 kg. In addition, insulin sensitivity (as determined by a hyperinsulinaemic euglycaemic clamp) almost doubled, and insulin secretion capacity (measured using a 30 mmol/l glucose clamp + arginine) greatly improved. There was no significant difference between results obtained after one and six weeks of treatment, but there was a tendency towards further improvement in plasma glucose as well as insulin secretion. There were few side effects and no differences between saline- and GLP-1-treated patients in this respect. Of note, the dose selected was not necessarily maximal (and was not associated with side effects). Further studies using the same technique indicated that a higher infusion rate might be even more effective [33].

The conclusion drawn was that GLP-1-based therapy has unusually attractive potential in diabetes treatment. Therefore, two strategies have been pursued: the development of DPP-IV-resistant analogues of GLP-1 and development of inhibitors of DPP-IV.

Resistant analogues or activators of the GLP-1 receptor

Exendin 4

DPP-IV cleaves peptides at the penultimate N-terminal amino acid residue if this is Pro or Ala (Ala in GLP-1). Therefore, substitution of this residue can render the molecule resistant [34]. However, this only prolongs the half-life of the molecule from 2 min to 4–5 min, because renal extraction and degradation effectively clears the plasma of substituted, as well as unsubstituted, GLP-1 [35]. A prolonged effect therefore requires changes that decrease renal elimination. Exendin 4, isolated from the saliva of the lizard *Heloderma suspectum* (also called the Gila monster) is such a molecule. It is 53% homologous to GLP-1 (but is not the GLP-1 of the Gila monster) and

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is cleared from plasma at a rate of 1.8 ml/kg/min, which is similar in magnitude to the normal glomerular filtration rate [36]. Otherwise, exendin 4 appears to act in humans in a manner identical to that of GLP-1 [36]. The clinical usefulness of exendin 4 was evaluated in a proof-of-concept Phase II study recently reported by the Amylin Corporation [37]. Exendin 4 (now named AC2993 or Exenatide) was injected subcutaneously twice or three times daily for four weeks in patients already treated with metformin, SU or both. In all groups, there was a reduction in HbA1c ranging from 0.7% to 1.1%. The most common adverse effect was transient mild to moderate nausea. Mild hypoglycaemia was reported in about a third of the patients also treated with SU. This finding was not substantiated by measurements, but could reflect a partial uncoupling of the glucose dependency of the insulinotropic actions of GLP-1 by SU (as discussed above) but, conversely, also illustrates the potency of this combination. In late 2003, the company completed Phase III studies with a similar design, in which Exenatide was given as twice-daily injections initially in doses of 5 μg for one month, and subsequently at 5 μg or 10 μg per injection for five months. This approach reduced the tendency to cause initial nausea. Mild hypoglycemia was noted in 35% of the patients also treated with SU. The average drop in HbA1c over six months of treatment was 1% from a base line value >8%, and values below 7% (the currently recommended target) were observed in 40–46% of patients (at 10 μg). Antibodies against Exenatide were observed in approximately one-fifth of the patients, but this was unrelated to the clinical efficacy. Recent studies presented by the Amylin Corporation at the American Diabetes Association (ADA) in Orlando 2004 indicated that subcutaneous injections of a stable GLP-1 receptor agonist (exendin 4) twice-daily for a year to individuals with T2DM were associated with a gradual weight loss, with no signs of impaired efficacy over time. Indeed, the efficacy of this appetite-reducing effect was demonstrated convincingly not only in these clinical studies but also in recent studies involving lifetime administration of exendin 4 to rats. The treated animals survived longer than controls, an effect that was thought to result from decreased food intake and hence a significantly lower body weight [38].

It can be concluded that Exenatide provides considerable additional glycemic control, even in patients inadequately treated with oral antidiabetic agents, and also causes weight loss, which can be predicted to provide further improvements of metabolism. However, two injections of exenatide per day do not provide a full 24-hour exposure to the GLP-1 receptor agonist, which is considered important for the full anti-diabetic effect of intravenously administered GLP-1 [39]; this might explain a less conspicuous effect on fasting plasma glucose.

Albumin-bound GLP-1 derivatives

Another approach has been to bind a GLP-1 analogue to albumin to exploit the slow elimination kinetics of this molecule in the body. Three different methods have been employed to achieve this: NovoNordisk in Denmark developed an acylated derivative of GLP-1 that binds non-covalently to albumin; the Canadian company, Conjuchem, created an analogue of GLP-1 which, after injection, establishes a covalent bond with albumin; and the American company Human Genome Sciences has generated a fusion protein consisting of a DPP-IV-resistant GLP-1 analogue covalently bound to human albumin.

NN2211

The selection of the NovoNordisk compound NN2211 for clinical development was recently described [40]. It consists of native GLP-1, in which a C16 acyl chain is attached via a glutamoyl spacer to Lys26 (Lys34 was substituted by Arg). The compound shows a slow release from the subcutaneous injection site and binds to albumin, which renders the molecule resistant to DPP-IV and allows at least the bound fraction to escapes renal elimination. This resulted in a half-life in healthy subjects and patients with T2DM of 10–12 h following a single subcutaneous injection [41] and thereby adequate 24 h exposure after a single daily injection. In addition, in chronic treatment, the large post-injection concentration excursions caused by less long-lived analogues that might be associated with side effects such as nausea are likely to be avoided, as shown in studies in pigs [42]. The analogue itself is equipotent to GLP-1 at the cloned human GLP-1 receptor. In clinical studies, NN2211 effectively reduced fasting, as well as meal-related (12 h post-injection) glycaemia, by modifying insulin secretion, delaying gastric emptying and suppressing prandial glucagon secretion [41] and, in a one-week study, improved both α and β cell function and reduced hepatic glucose production [43]. Phase II studies involving three months of daily injections have recently been reported [44]. In a blinded design, ascending doses of NN2211 in monotherapy were compared with glimepiride. It was concluded that NN2211 improved glycaemic control significantly and was comparable to glimepiride. Weight was maintained with a tendency to decrease, and the risk of hypoglycemica was low. It is noteworthy that antibodies against NN2211 could not be detected. This analogue clearly possesses favorable pharmacokinetic properties.

CJC-1131

The Conjuchem compound CJC-1131 is composed of a D-Ala8-substituted GLP-1 molecule with a linker and a reactive moiety (maleimidoproprionic acid) attached to the C-terminus. After injection *in vivo*, this molecule conjugates covalently to Lys34 of the albumin molecule and thereby acquires the half-life of albumin. The CJC-1131–albumin conjugate binds to the GLP-1 receptor and

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activates cAMP with a potency similar to that of GLP-1 [45]. In recent studies in human volunteers, elimination half-lives ranging from 9–14 days were noted [46], and in studies presented at the International Diabetes Federation Congress in 2003 the compound was reported to have dose-dependent effects on glycaemia and body weight lasting at least 48 h, and up to eight days in some patients. Results of Phase II clinical studies have recently (July 2004) been announced as a press release from the company and, although effective in reduced fasting blood glucose and HbA1c levels as well as body weight, the compound was most effective when administered once daily, contrasting with the reported long half-life. The company claims that the conjugate is not antigenic.

Albugon

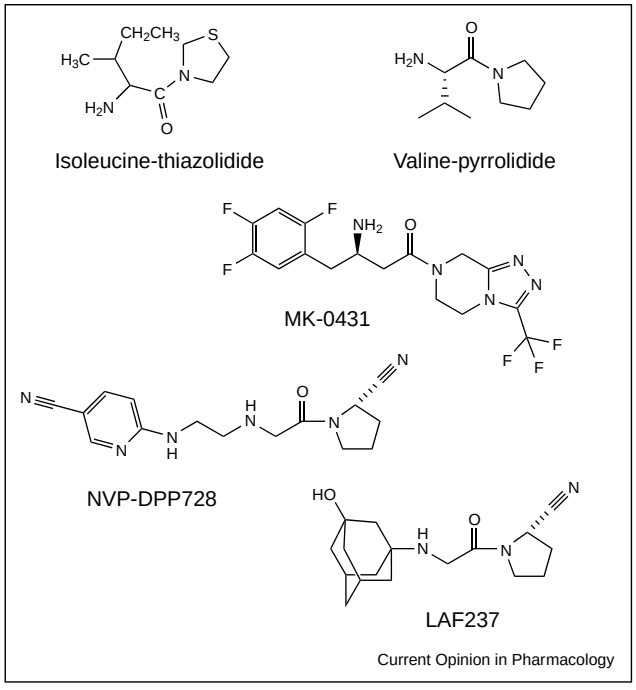
Very little is known about the Albugon compound from Human Genome Sciences. However, it has been reported to retain the insulinotropic activities of GLP-1 and to delay gastric emptying. In glucose-intolerant mice and in diabetic rats, a single injection almost normalized glucose levels for 24 h; the half life was said to be three days in monkeys [47].

Inhibitors of DPP-IV

Preclinical studies

Therapeutic use of inhibitors of the enzyme responsible for the inactivation of GLP-1 as anti-diabetic agents was first proposed in 1995 [48] on the basis of the finding that GLP-1 seems uniquely sensitive to cleavage by DPP IV; compounds of this class have now reached Phase III clinical trials. With a DPP-IV inhibitor (see Figure 1), it is possible to completely prevent the N-terminal degradation of GLP-1 that occurs *in vivo*, resulting in significant enhancement of its insulinotropic activity [49]. Studies in Vancouver diabetic fatty rats have shown that chronic oral administration of the Probiodrug DPP-IV inhibitor isoleucine thiazolidide (P32/98) for 12 weeks improves glucose tolerance, insulin sensitivity and β-cell responsiveness [50]. The longer-acting Ferring inhibitor, FE 999-011, continuously inhibits plasma DPP IV activity and not only normalises the glucose excursion after oral glucose administration in insulin-resistant Zucker obeses rats but also delays the onset of hyperglycaemia in Zucker diabetic fatty rats [51]. These effects were, at least in part, attributed to increased levels of intact GLP-1. Increased intact GLP-1 concentrations were also implicated in the improved islet function seen after chronic treatment of high-fat fed (glucose-intolerant and insulin-resistant) mice with valine-pyrrolidide [52]. Fischer rats, which have a catalytically inactive DPP-IV molecule, and CD26 knockout mice with a targeted disruption of the gene encoding DPP-IV further support the involvement of DPP-IV in mediating glucose tolerance. Such animals have improved glucose tolerance compared with their wild-type counterparts [53–55]. In DPP-IV-negative Fischer rats and DPP-IV inhibitor-treated control animals, the impaired glucose tolerance that normally develops with ageing is prevented [55,56], whereas the lack of DPP-IV protects both Fischer rats and CD26 knockout mice from diet (high fat)-induced insulin resistance and glucose intolerance [56–58]. Again, these effects are believed to involve preservation of endogenous GLP-1 levels, because intact GLP-1 concentrations are elevated.

Figure 1



Structures of DPP-IV inhibitors.

Clinical studies

After these promising preclinical studies, the first clinical proof-of-concept was obtained using the short-acting Novartis inhibitor, NVP-DPP728 [59]. When given twice or three times daily for four weeks in patients with relatively mild T2DM (mean HbA1c of 7.4%), both fasting and prandial glucose levels were lowered significantly, resulting in a reduction in HbA1c of 0.5%; despite the fall in glycemia, fasting and post-prandial insulin levels were sustained. NVP-DPP728 appeared to be well tolerated, with only minor adverse events being reported. However, some of these symptoms (pruritus and nasopharyngitis) seem to be drug- rather than class-specific, because they were not reported for another inhibitor, LAF237, also developed by Novartis. NVP-DPP728 has now been dropped in favour of LAF237, which is longer-acting and suitable for once-daily administration. A clinical study with this compound was recently reported, showing it to have a pharmacodynamic profile similar to that of its predecessor [60]. The mechanism of

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action was suggested to be incretin-mediated, because LAF237 treatment increased both baseline and prandial active GLP-1 levels. As with NVP-DPP78, insulin levels were not increased but, interestingly, glucagon levels were significantly suppressed. Clinical data from longer-term studies presented at the recent ADA meeting in Orlando showed that 12 weeks of monotherapy with LAF237 was associated with sustained reductions in HbA1c (from a starting level of 8%, falling to 7.4% at the end of the study) [61]. Encouragingly, patients with the worst metabolic control (HbA1c ranging between 8% and 9.5%) showed the greatest reductions (1.2%), suggesting that DPP-IV inhibition may not be restricted only to those patients with mild diabetes. Furthermore, LAF237 was able to prevent the worsening of glycaemic control when given for up to 12 months in combination with metformin in patients otherwise inadequately controlled with metformin alone [62]. Side effects were mild and, importantly, hypoglycemia was not reported. However, in contrast to GLP-1 analogues, there was no change in body weight. Phase III clinical trials are currently in progress, and filing for FDA approval is expected in 2006.

Merck also has an inhibitor (MK-0431) in Phase III trials (http://www.merck.com), but so far little is known about this compound. Results of placebo-controlled, single-dose studies were presented at the ADA in Orlando. MK-0431 was well tolerated and caused significant reductions in the glycaemic excursion following an oral glucose tolerance test, which were associated with increases in intact GLP-1 and insulin, and reductions in glucagon secretion [63].

DPP-IV inhibitors are in development at GlaxoSmithKline (Phase I), Bristol-Meyer-Squibb (Phase II) and Probiodrug (P93/01; Phase II), with several other companies reportedly having a DPP-IV inhibitor programme. Single doses of P93/01 were shown to have good tolerability and result in dose-related reductions in prandial glucose in T2DM subjects when HbA1c was above 6% [64].

The clinical studies with DPP IV inhibitors that have been reported so far have not been associated with any serious adverse side effects, but there has been understandable concern that undesirable side effects could arise from inhibiting an enzyme with multiple substrates or because of non-mechanism-based actions (i.e. not related to the selective inhibition of DPP-IV). With regard to multiple substrates, although several regulatory peptides, neuropeptides, chemokines and cytokines have been identified as potential substrates from *in vitro* kinetic studies (reviewed by Lambeir *et al.* [65•]), it is uncertain how many of these are endogenous substrates and, if so, whether DPP-IV-mediated degradation is their primary route of elimination. In addition to GLP-1, the other incretin hormone, glucose-dependent insulinotropic polypeptide, is an endogenous DPP-IV substrate, as is the neuropeptide pituitary adenylate cyclase-activating peptide [66], but inhibition of their degradation would be expected to contribute to the anti-diabetic effects of DPP-IV inhibitors. The evidence for a physiological role for DPP-IV in degradation of many of the other potential substrates remains to be demonstrated. DPP-IV also has several other roles that could potentially be compromised by DPP-IV inhibition. It is present on the surface of T cells (where it is usually referred to as the T cell marker CD26) and contributes to T cell activation and proliferation via its interaction with other membrane-expressed molecules such as CD45, although it is uncertain whether the catalytic activity is required, or indeed whether its presence is obligatory [67]. In this context, a family of DPP-IV-related enzymes is now known to exist, which have similar catalytic activities. Selective inhibition of two of these enzymes (DPP 8 and DPP 9) was recently reported to affect T cell activation *in vitro* [68] and be associated with severe, even lethal side effects in preclinical species [69], whereas selective DPP-IV inhibition was not, suggesting that DPP 8 and 9 could be responsible for some of the functions previously attributed to DPP-IV. In turn, this raises the possibility that some of the potential or reported side effects of DPP IV inhibition could be attributable to inhibition of DPP 8 and 9, rather than DPP-IV itself. It is, therefore, highly relevant that rodents which lack DPP-IV enzymatic activity (the Fischer rat and the CD26 knockout mouse) are completely viable and seem to suffer no ill effects because of the lack of DPP-IV. Selectivity data for the inhibitors in development have not been released, apart from the Merck compound, which is reported to have >2500-fold selectivity for DPP-IV relative to DPP 8 and 9 [70].

Conclusions

It seems clear from the most recent clinical results that both GLP-1 analogues (or GLP-1 receptor activators such as exendin) and DPP-IV inhibitors effectively improve metabolic control in patients with T2DM. Both seem to be effective in monotherapy and in combination with other antidiabetic agents. DDP-IV inhibitors are administered orally, whereas the analogues require parenteral administration. The analogues cause significant reductions in body weight, whereas the inhibitors seem to be weight neutral. Clinical data obtained so far do not allow conclusions to be drawn on whether the protective effect on β-cells seen in laboratory animals can also be demonstrated in patients with T2DM.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Holst JJ, Gromada J: **Role of incretin hormones in the regulation of insulin secretion in diabetic and nondiabetic humans**. *Am J Physiol Endocrinol Metab* 2004, **287**:E199-E206.

2. Nauck M, Stockmann F, Ebert R, Creutzfeldt W: **Reduced incretin effect in type 2 (non-insulin-dependent) diabetes**. *Diabetologia* 1986, **29**:46-52.

3. Vilsboll T, Holst JJ: **Incretins, insulin secretion and type 2 diabetes mellitus**. *Diabetologia* 2004, **47**:357-366.

4. Kjems LL, Holst JJ, Volund A, Madsbad S: **The influence of GLP-1 on glucose-stimulated insulin secretion: effects on beta-cell sensitivity in type 2 and nondiabetic subjects**. *Diabetes* 2003, **52**:380-386.

5. Vilsboll T, Knop FK, Krarup T, Johansen A, Madsbad S, Larsen S, Hansen T, Pedersen O, Holst JJ: **The pathophysiology of diabetes involves a defective amplification of the late-phase insulin response to glucose by glucose-dependent insulinotropic polypeptide-regardless of etiology and phenotype**. *J Clin Endocrinol Metab* 2003, **88**:4897-4903.

6. 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**:205-211.

7. 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**:1316-1322.

8. Holst JJ: **Therapy of type 2 diabetes mellitus based on the actions of glucagon- like peptide-1**. *Diabetes Metab Res Rev* 2002, **18**:430-441.

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

10. Buteau J, Roduit R, Susini S, Prentki M: **Glucagon-like peptide-1 promotes DNA synthesis, activates phosphatidylinositol 3-kinase and increases transcription factor pancreatic and duodenal homeobox gene 1 (PDX-1) DNA binding activity in beta (INS-1)-cells**. *Diabetologia* 1999, **42**:856-864.

11. Egan JM, Bulotta A, Hui H, Perfetti R: **GLP-1 receptor agonists are growth and differentiation factors for pancreatic islet beta cells**. *Diabetes Metab Res Rev* 2003, **19**:115-123.

12. 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**:2270-2276.

13. Stoffers DA, Kieffer TJ, Hussain MA, Drucker DJ, Bonner-Weir S, Habener JF, Egan JM: **Insulinotropic glucagon-like peptide 1 agonists stimulate expression of homeodomain protein IDX-1 and increase islet size in mouse pancreas**. *Diabetes* 2000, **49**:741-748.

14. Zhou J, Wang X, Pineyro MA, Egan JM: **Glucagon-like peptide 1 and exendin-4 convert pancrteatic AR42J cells into glucagon- and insulin-producing cells**. *Diabetes* 1999, **48**:2358-2366.

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

16. •• Buteau J, El-Assaad W, Rhodes CJ, Rosenberg L, Joly E, Prentki M: **Glucagon-like peptide-1 prevents beta cell glucolipotoxicity**. *Diabetologia* 2004, **47**:806-815.
Demonstrates the powerful protective effects of GLP-1 on apoptosis induced by lipo- or gluco-toxicity in human β-cells.

17. Toft-Nielsen MB, Damholt MB, Madsbad S, Hilsted LM, Hughes TE, Michelsen BK, Holst JJ: **Determinants of the impaired secretion of glucagon-like peptide-1 in type 2 diabetic patients**. *J Clin Endocrinol Metab* 2001, **86**:3717-3723.

18. 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**:665-673.

19. Nauck MA, Niedereichholz U, Ettler R, Holst JJ, Orskov C, Ritzel R, Schmiegel WH: **Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans**. *Am J Physiol* 1997, **273**:E981-E988.

20. Young A, Denaro M: **Roles of amylin in diabetes and in regulation of nutrient load**. *Nutrition* 1998, **14**:524-527.

21. Verdich C, Flint A, Gutzwiller JP, Naslund E, Beglinger C, Hellstrom PM, Long SJ, Morgan LM, Holst JJ, Astrup A: **A meta-analysis of the effect of glucagon-like peptide-1 (7-36) amide on ad libitum energy intake in humans**. *J Clin Endocrinol Metab* 2001, **86**:4382-4389.

22. Bullock BP, Heller RS, Habener JF: **Tissue distribution of messenger ribonucleic acid encoding the rat glucagon-like peptide-1 receptor**. *Endocrinology* 1996, **137**:2968-2978.

23. • Gros R, You X, Baggio LL, Kabir MG, Sadi AM, Mungrue IN, Parker TG, Huang Q, Drucker DJ, Husain M: **Cardiac function in mice lacking the glucagon-like peptide-1 receptor**. *Endocrinology* 2003, **144**:2242-2252.
Demonstrates that heart function is impaired in the absence of GLP-1.

24. •• Bose KA, Mocanu MM, Carr RD, Brand CL, Yellon DM: **Glucagon-like peptide-1(GLP-1) can directly protect the heart against ischemia/reperfusion injury**. *Diabetes* 2004, in press.
Demonstrates clinical improvement of cardiac function with GLP-1 in patients with cardiac failure after myocardial infarction and angioplasty.

25. Nikolaidis LA, Mankad S, Sokos GG, Miske G, Shah A, Elahi D, Shannon RP: **Effects of glucagon-like peptide-1 in patients with acute myocardial infarction and left ventricular dysfunction after successful reperfusion**. *Circulation* 2004, **109**:962-965.

26. Yamamoto H, Lee CE, Marcus JN, Williams TD, Overton JM, Lopez ME, Hollenberg AN, Baggio L, Saper CB, Drucker DJ, Elmquist JK: **Glucagon-like peptide-1 receptor stimulation increases blood pressure and heart rate and activates autonomic regulatory neurons**. *J Clin Invest* 2002, **110**:43-52.

27. Yamamoto H, Kishi T, Lee CE, Choi BJ, Fang H, Hollenberg AN, Drucker DJ, Elmquist JK: **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**:2939-2946.

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**:824-830.

29. 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**:881-888.

30. During MJ, Cao L, Zuzga DS, Francis JS, Fitzsimons HL, Jiao X, Bland RJ, Klugmann M, Banks WA, Drucker DJ, Haile CN: **Glucagon-like peptide-1 receptor is involved in learning and neuroprotection**. *Nat Med* 2003, **9**:1173-1179.

31. • Perry TA, Greig NH: **A new Alzheimer's disease interventive strategy: GLP-1**. *Curr Drug Targets* 2004, **5**:565-571.
This review summarizes the evidence that GLP-1 might also have neuroprotective effects.

32. Vilsboll T, Agerso 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**:220-224.

33. Ehlers MRW, Roderick EH, Schneider RL, Kipnes MS: **Continuous subcutaneous infusion of recombinant GLP-1 for 7 days dose-dependently improved glycemic controls in type 2 diabetes [abstract]**. *Diabetes* 2002, **51 (Suppl 2)**:A579.

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34. 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**:271-278.

35. Meier JJ, Nauck MA, Kranz D, Holst JJ, Deacon CF, Gaeckler D, Schmidt WE, Gallwitz B: **Secretion, degradation, and elimination of glucagon-like peptide 1 and gastric inhibitory polypeptide in patients with chronic renal insufficiency and healthy control subjects**. *Diabetes* 2004, **53**:654-662.

36. Edwards CM, Stanley SA, Davis R, Brynes AE, Frost GS, Seal LJ, Ghatei MA, Bloom SR: **Exendin-4 reduces fasting and postprandial glucose and decreases energy intake in healthy volunteers**. *Am J Physiol Endocrinol Metab* 2001, **281**:E155-E161.

37. Fineman MS, Bicsak TA, Shen LZ, Taylor K, Gaines E, Varns A, Kim D, Baron AD: **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**:2370-2377.

38. Hiles R, Carpenter T, Serota D, Schafer K, Ross P, Nelsen D, Rebelatto M: **Exenatide does not cause pancreatic tumours or malignancies in rats and mice following a 2-year period of exposure [abstract]**. *Diabetes* 2004, **53 (Suppl 2)**:A380.

39. Larsen J, Hylleberg B, Ng K, Damsbo P: **Glucagon-like peptide-1 infusion must be maintained for 24 h/day to obtain acceptable glycemia in type 2 diabetic patients who are poorly controlled on sulphonylurea treatment**. *Diabetes Care* 2001, **24**:1416-1421.

40. Knudsen LB, Agersø H, Bjenning C, Bregenholt S, Gotfredsen C, Holst JJ, Huusfeldt PO, Larsen MØ, Larsen PJ, Nielsen PF *et al.*: **GLP-1 derivatives as novel compounds for the treatment of type 2 diabetes**. *Drugs of the Future* 2001, **26**:677-685.

41. Juhl CB, Hollingdal M, Sturis J, Jakobsen G, Agerso H, Veldhuis J, Porksen N, Schmitz O: **Bedtime administration of NN2211, a long-acting GLP-1 derivative, substantially reduces fasting and postprandial glycemia in type 2 diabetes**. *Diabetes* 2002, **51**:424-429.

42. Ribel U, Larsen MO, Rolin B, Carr RD, Wilken M, Sturis J, Westergaard L, Deacon CF, Knudsen LB: **NN2211: a long-acting glucagon-like peptide-1 derivative with anti-diabetic effects in glucose-intolerant pigs**. *Eur J Pharmacol* 2002, **451**:217-225.

43. Degn KB, Juhl CB, Sturis J, Jakobsen G, Brock B, Chandramouli V, Rungby J, Landau BR, Schmitz O: **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**:1187-1194.

44. Madsbad S, Schmitz O, Ranstam J, Jakobsen G, Matthews DR: **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**:1335-1342.

45. Kim JG, Baggio LL, Bridon DP, Castaigne JP, Robitaille MF, Jette L, Benquet C, Drucker DJ: **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**:751-759.

46. Lawrence B, Dreyfus J, Wen S, Guicarc'h P, Drucker D, Castaigne JP: **CJC-II31, a long acting GLP-1 derivative, exhibits an extended pharmacokinetic profile in healthy human volunteers [abstract]**. *Diabetes* 2003, **52 (Suppl 1)**:A125.

47. Bloom M, Bock J, Duttaroy A, Grzegorzewski K, Moor P, Ou Y, Wojcik S, Zhou X, Bell A: **Albugon fusion protein: A long acting analogue of GLP-1 that provides lasting antidiabetic effect in animals [abstract]**. *Diabetes* 2003, **52 (Suppl 1)**:A112.

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

49. Deacon CF, Hughes TE, Holst JJ: **Dipeptidyl peptidase IV inhibition potentiates the insulinotropic effect of glucagon-like peptide 1 in the anesthetized pig**. *Diabetes* 1998, **47**:764-769.

50. Pospisilik JA, Stafford SG, Demuth HU, Brownsey R, Parkhouse W, Finegood DT, McIntosh CH, Pederson RA: **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**:943-950.

51. Sudre B, Broqua P, White RB, Ashworth D, Evans DM, Haigh R, Junien JL, Aubert ML: **Chronic inhibition of circulating dipeptidyl peptidase IV by FE 999011 delays the occurrence of diabetes in male zucker diabetic fatty rats**. *Diabetes* 2002, **51**:1461-1469.

52. 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**:717-727.

53. Nagakura T, Yasuda N, Yamazaki K, Ikuta H, Yoshikawa S, Asano O, Tanaka I: **Improved glucose tolerance via enhanced glucose-dependent insulin secretion in dipeptidyl peptidase IV-deficient Fischer rats**. *Biochem Biophys Res Commun* 2001, **284**:501-506.

54. Marguet D, Baggio L, Kobayashi T, Bernard AM, Pierres M, Nielsen PF, Ribel U, Watanabe T, Drucker DJ, Wagtmann N: **Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26**. *Proc Natl Acad Sci USA* 2000, **97**:6874-6879.

55. Mitani H, Takimoto M, Kimura M: **Dipeptidyl peptidase IV inhibitor NVP-DPP728 ameliorates early insulin response and glucose tolerance in aged rats but not in aged Fischer 344 rats lacking its enzyme activity**. *Jpn J Pharmacol* 2002, **88**:451-458.

56. Mitani H, Takimoto M, Hughes TE, Kimura M: **Dipeptidyl peptidase IV inhibition improves impaired glucose tolerance in high-fat diet-fed rats: study using a Fischer 344 rat substrain deficient in its enzyme activity**. *Jpn J Pharmacol* 2002, **88**:442-450.

57. Yasuda N, Nagakura T, Yamazaki K, Inoue T, Tanaka I: **Improvement of high fat-diet-induced insulin resistance in dipeptidyl peptidase IV-deficient Fischer rats**. *Life Sci* 2002, **71**:227-238.

58. Conarello SL, Li Z, Ronan J, Roy RS, Zhu L, Jiang G, Liu F, Woods J, Zycband E, Moller DE *et al.*: **Mice lacking dipeptidyl peptidase IV are protected against obesity and insulin resistance**. *Proc Natl Acad Sci USA* 2003, **100**:6825-6830.

59. Ahren B, Simonsson E, Larsson H, Landin-Olsson M, Torgeirsson H, Jansson PA, Sandqvist M, Bavenholm P, Efendic S, Eriksson JW *et al.*: **Inhibition of dipeptidyl peptidase IV improves metabolic control over a 4-week study period in type 2 diabetes**. *Diabetes Care* 2002, **25**:869-875.

60. 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**:2078-2084.

61. Pratley RE, Galbreath E: **Twelve-week monotherapy qwith the DPP-4 inhibitor, LAF 237 improves glycemic control in patients with type 2 diabetes (T2DM) [abstract]**. *Diabetes* 2004, **53(Suppl 2)**:A83.

62. Ahren B, Gomis R, Standl E, Mills D, Schweizer A: **Prolonged efficacy of LAF237 in patients with type 2 diabetes (T2DM) inadequately treated with metformin [abstract]**. *64th Scientific Sessions of the American Diabetes Association 2004. Orlando, USA*.

63. Herman GA, Zhao PL, Dietrich B *et al.*: **The DP-IV inhibitor MK-0431 enhances active GLP-1 and reduces glucose following an OGTT in type 2 diabetics [abstract]**. *Diabetes* 2004, **53(Suppl 2)**:A82.

64. Heins J, Glund K, Hoffmann T, Metzner J, Demuth H-U: **The DP-IV inhibitor P93/01 improves glucose tolerance in humans with HbA1c gerater than 6.0 [abstract]**. *Diabetes* 2004, **53(Suppl 2)**:A128.

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65. Lambeir AM, Durinx C, Scharpe S, De Meester I:
• **Dipeptidyl-peptidase IV from bench to bedside: an update on structural properties, functions, and clinical aspects of the enzyme DPP IV**. *Crit Rev Clin Lab Sci* 2003, **40**:209-294.
A very useful and comprehensive review of the enzymology of DPP-IV.

66. Zhu L, Tamvakopoulos C, Xie D, Dragovic J, Shen X, Fenyk-Melody JE, Schmidt K, Bagchi A, Griffin PR, Thornberry NA, Sinha RR: **The role of dipeptidyl peptidase IV in the cleavage of glucagon family peptides: *in vivo* metabolism of pituitary adenylate cyclase activating polypeptide-(1-38)**. *J Biol Chem* 2003, **278**:22418-22423.

67. von Bonin A, Huhn J, Fleischer B: **Dipeptidyl-peptidase IV/CD26 on T cells: analysis of an alternative T-cell activation pathway**. *Immunol Rev* 1998, **161**:43-53.

68. Leiting B, Nichols E, Biftu T, Edmonson S, Ok H, Weber AE, Zaller D, Thornberry NA: **Inhibition of dipeptidyl peptidase IV does not attenuate T cell activation *in vitro* [abstract]**. *Diabetes* 2004, **53 (Suppl 2)**:A2.

69. Lankas G, Leiting B, Roy RS, Eiermann G, Biftu T, Kim D, Ok H, Weber AE, Thornberry NA: **Inhibition of DPP8/9 results in toxicity in preclinical species: potential importance of selective dipeptidyl peptidase IV ionhibition for the treatment of type 2 DM (abstract]**. *Diabetes* 2004, **53 (Suppl 2)**:A2.

70. Weber AE, Kim D, Beconi M *et al.*: **MK-0431 is a potent, selective dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes [abstract]**. *Diabetes* 2004, **53 (Suppl 2)**:A151.

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