

Glucagon-like peptide-1 derivatives and dipeptidyl peptidase-IV inhibitors

New hope for the treatment of type-2 diabetes

Mohamed A. Al-Omar, PhD, Mohammed N. Al-Arifi, PhD.

ABSTRACT

Glucagon-like peptide (GLP-1) is an endogenous insulinotropic/glucagonostatic hormone that acts in a self-limiting mechanism. It is a multifunctional hormone that leads to insulin release stimulation, liver glucagon breakdown suppression, upregulation of islet cell proliferation, and neogenesis and retardation of gastric emptying. The short half-life and high renal clearance due to degradation via dipeptidyl peptidase-IV (DPP-IV), and active glomerular filtration rate make this hormone ineffectual as an exogenous agent. More stable and long acting GLP-1 analogues and DPP-1 inhibitors have been developed with promising clinical value for the treatment of type-2 diabetes. The GLP-1 derivatives have the advantage of decreasing body weight while the DPP-IV inhibitors can be administered orally up to once daily. The mechanism of action as well as the tolerable side effect is astounding. This review article covers this new generation of anti-diabetics.

Saudi Med J 2005; Vol. 26 (10): 1511-1515

Glucagon-like peptide (GLP-1) was discovered 25 years ago in the Laboratory of Molecular Endocrinology, Massachusetts General Hospital. Three years later Bell et al,¹ isolated the first mammalian cDNA, which consists of 3 glucagon-related peptides, and this is what Dr. Bell and his team termed GLP-1. One year later, Bell et al,² isolated the human preproglucagon cDNA and, the next year, Heinrich et al,³ characterized rat cDNA. The GLP-1 is a 30 amino acid peptide secreted from the distal intestinal L-cells after ingestion of carbohydrate and fat.^{4,5} It has a hypoglycemic action called the incretion effect, which was reduced or even absent in patients with type-2 diabetes. This incretion hormone is a multifunctional hormone, and this explains its potential use as an anti-hyperglycemic agent. The GLP-1 stimulates insulin

release and suppresses glucagon secretion in a glucose-dependent manner.^{6,7} It also activates insulin gene transcription, β -cell proliferation (hyperplasia), and neogenesis, which is mediated by upregulation of the pancreatic duodenal homeobox-1 (PDX-1) transcription factor.^{8,9} The GLP-1 and its dipeptidyl peptidase-IV (DPP-IV) resistant analogues, a class of agents designed to maintain affinity of the analogues to GLP-1 receptors as does GLP-1 itself, stimulate glucose-dependent insulin release, and suppress glucagon secretion, retard gastric motility, inhibit appetite, and food intake, enhance their ability to activate insulin gene transcription, β -cells growth and neogenesis, and at the same time to be resistance to degradation by DPP-IV enzyme. Recently, Buteau et al¹⁰ reported that GLP-1 inhibits

From the Department of Pharmaceutical Chemistry (Al-Omar) and the Director of Drug and Poison Information Center (Al-Arifi), College of Pharmacy, King Saud University, Riyadh, Kingdom of Saudi Arabia.

Address correspondence and reprint request to: Dr. Mohammed N. Al-Arifi, Director of Drug and Poison Information Center, College of Pharmacy, King Saud University, PO Box 2457, Riyadh 11451, Kingdom of Saudi Arabia. Tel. +966 (1) 4677352. Fax. +966 (1) 4676229. E-mail: malarifi@ksu.edu.sa

apoptosis of β -cells. The net result of the last 2 actions can lead to increase in β -cells mass and number. The GLP-1 also decelerates the gastric emptying process, suppresses appetite, and induces satiety, leading to weight loss after several weeks of administration.^{11,12} Finally, it may improve cellular sensitivity to insulin or the insulinotropic effect in type-2 diabetes.¹³ In human, GLP-1 has cardiovascular and neuroprotective effects that are independent of insulin effect, and may protect the ischemic and reperfused myocardium and brain.^{14,15}

Mechanism of action of GLP-1. The GLP-1 acts as an incretin hormone that stimulates insulin release in the presence of nutrients in a glucose-dependent manner.^{6,7} The stimulation of insulin secretion after oral administration of nutrients was more potent than intravenously.¹⁶ This is due to the incretin effect on GLP-1, which in turn stimulates insulin release after each meal. In agreement with this, intravenous infusions of GLP-1 have been shown to decrease glucose level in type-2 diabetes.¹⁶ Binding of GLP-1 to its receptor on β -cells leads to activation of adenylate cyclase via G-protein, resulting in the formation of cAMP and then activation of a cascade of events that enhance secretion of insulin from its granule.¹⁶ Interestingly, all type-2 diabetic patients at any stage of their disease respond to GLP-1-derivative treatment.¹⁷

Metabolism and excretion of GLP-1. The GLP-1 has a short half-life of 1-2 minutes that abridged the therapeutic effect of this agent. The GLP-1 is extensively and rapidly degraded by an ubiquitous enzyme called DPP-IV, EC 3.4.14.5.¹⁸ This proteolytic enzyme removes 2 amino acids (His-7-Ala-8) from the N-terminus resulting in formation of inactive GLP-1 (Glu9 Gly37).¹⁹ Another withdrawing factor is the rapid renal clearance (see Exendin 4).¹⁸

Therapeutic agents related to GLP-1. Two main strategies have been followed to augment the physiological and pharmacological action of GLP-1, and the subsequent insulin secretion. The first is the development of DPP-IV resistant analogues of GLP-1, and the second strategy depends on the development of DPP-IV inhibitors.

Exendin-4. The Ala-8 at the N-terminal amino acid residue of GLP-1 peptide is very sensitive to degradation by DPP-IV. Replacement of this residue can render the molecular resistant to the inactivation process.²⁰ Exendin-4, is a 39 residue peptide, and it has been isolated from the venom of gila monster (*Heloderma suspectum*), and shares a 53% sequence homology with GLP-1.²¹ This molecule has a long half-life of approximately 3 hours and is eventually cleared by the kidney at a rate of 1.8 ml/kg/min, which keeps plasma concentrations at normal GLP-1 level if the drug is administered subcutaneously twice or thrice daily.²² Binding of GLP-1 analogues

to albumin will decrease the renal elimination of the compounds to normal albumin clearance rate (see liraglutide).²³ The main side effects of exendin-4 include nausea, vomiting, and mild headache, however, a new regimen has been produced to decrease the side effects, where exendin-4 was injected twice daily initially in dose of 5 μ g for one month and followed by 10 μ g for 5 months.²⁴ Due to the protein nature of exendin-4, a concern about antigen-antibody reaction in human has arisen. However, more than 25% of patients have developed antibodies against the drug, but this was not associated with any decrease in both the pharmacological and physiological efficacy of exendin-4 and native GLP-1.²² A recent study indicated that subcutaneous injection of the drug to type-2 diabetics were associated with appetite suppression and subsequent weight loss over a year of twice daily administration.²⁴ It can be concluded that monotherapy with exendin-4 can control blood glucose of patients with type-2 diabetes in a glucose-dependent manner and also can lead to body weight loss.

ZP-10. The ZP-10 is a modified GLP-1 peptide with a tail of lysines at the N-terminus. This drug is in the clinical testing phase with promising effects. It has similar insulinotropic action on β -cells. Limited information is available for this product.²⁵

Other GLP-1 derivatives that bind to serum albumin. While the exendin-4 molecule shares only 53% homology with a native GLP-1 the rest of GLP-1 analogues are identical to GLP-1, but they are protected from DPP-IV, and extensive renal clearance by different strategies, in which the analogue binds to a serum albumin after their absorption. Three new modified GLP-1 analogues, which binds to albumin have been developed: The first is liraglutide, an acylated derivative of GLP-1 where a 16-carbon fatty acid chain interacts non-covalently with albumin after injection.²⁶ The second derivative is CJC-1131, which binds to albumin in a covalent bond at the Lys31 amino acid.²⁷ The third drug albugon, also binds covalently to albumin.²⁸ Recently, Liu et al,²⁹ showed that N-terminal modification of GLP-1 by acetylation retaining GLP-1 activities had improved effects on pancreatic β -cell expression. Other modifications on N-terminus including N-glycation have been shown to improve resistance to DPP-IV whilst rendering insulinotropic action on β -cells.³⁰

Liraglutide (NN2211). Liraglutide (NN2211) has been introduced to treat type-2 diabetics. This derivative has the same pharmacological action and side effects as GLP-1, but with long half-life of approximately 11 hours after subcutaneous injection, which allows once daily administration.³¹ It reduces HbA1c up to 0.75% of baseline levels, 7.6% after 12 weeks of administration.³² In comparison to

metformin or glimepiride monotherapy, liraglutide lowers fasting blood glucose concentrations more efficiently.³³ While glimepiride can cause weight gain, liraglutide led to significant weight loss in conjunction with other GLP-1 effects.³³ In comparison to placebo, very few hypoglycemic episodes have been reported in the presence or absence of metformin treatment.³⁴

CJC-1131. The CJC-1131 is a GLP-1 analogue that is resistant to DPP-IV degradation. It differs from the native GLP-1 in 2 positions, firstly, amino acid number 2 has been replaced with D-ala instead of L-ala, and secondly, the attachment of albumin to maleimidopropionic acid moiety at position 31.²⁷ The half-life of this compound ranges from 9-14 days, allowing once-daily administration.³⁵ The CJC-1131 has the same therapeutic effects as GLP-1 with mild to moderate side effects.³⁵ Recent studies indicated, that the hypoglycemia, and body weight loss effect are dose-dependent, and the drug was more potent when injected once daily. Also, it had been claimed that CJC-1131 is not antigenic either to humans or to GLP-1.²⁴

Albugon. Albugon, is a long acting insulinotropic/glucagonostatic GLP-1 agonist with all physiological GLP-1 activities. Most of the investigations have been carried on animal including genetically modified mice, rats, and monkeys.²⁸ Albugon has been introduced recently by the American Company Human Genome Science. It is a long acting insulinotropic/glucagonostatic GLP-1 agonist with all physiological GLP-1 activities. Most of the investigations have been carried out on animals including genetically modified mice, rats, and monkeys.²⁸ Albugon was created using Human Genome Sciences' proprietary albumin fusion technology, which involves fusing the gene that expresses human albumin to the gene that expresses a therapeutically active protein. Research has shown that the fusion of therapeutic proteins to human albumin decreases clearance, and prolongs half-life. Albugon results from the genetic fusion of human albumin and GLP-1. The drug is currently in late-stage preclinical development. Human Genome Sciences will be responsible for the manufacture of albugon for phase 1 and 2 clinical trials.

DPP-IV inhibitors. Ten years ago, Deacon et al,³⁶ at the University of Copenhagen discovered DPP-IV. Once GLP-1 passes from L cells to intestinal capillaries, it encounters the proteolytic enzyme DPP-IV.¹⁶ This enzyme attacks GLP-1 at the N-terminus and inactivates the hormone. The truncated metabolites of this catalytic reaction are inactive and do not affect glucose or insulin levels.³⁷ This raises the notion of inhibition of DPP-IV as a strategy for new anti-diabetic agents. Pursuing DPP-IV inhibition has its risks as its inhibition could interact with its physiological functions. Other substrates for DPP-IV include neuropeptides,

chemokines, and white blood cell activators (DPP-IV is known within immunology- scientists as CD-26).³⁸ A pre-clinical study shows a toxic or lethal side effect as a result of non-selective inhibition of DPP-IV related enzymes including DPP-VIII and DPP-IX, which may explain some of unwanted effects.³⁹ In fact, animals that lack DPP-IV activities have no serious side effects during short run experiments. However, selective inhibition of DPP-IV is required as much as possible. Apart from MK-0431 (DDPIV inhibitor), which is reported to have a >2500-fold selectivity for DPP-IV relative to DPP-VIII, and DPP-IX had negligible effect or interaction with other types of DPP-IV inhibitors.³⁹ The most merit for DPP-IV inhibitor is the oral administration with all GLP-1 agonist activities. However, these inhibitors have no effect on body weight. Several compounds have been developed during the last decade including: LAF-237, NVP-DPP-728, P-93/01, P-32/98 (isoleucine thiazolidine), BMS-477188, MK-0431, and GSK-23A. (Figure 1) The LAF-237 was the first developed DPP-IV inhibitor. It is a valine pyrrolidine that fits very nicely into the enzyme active site and searches for smaller molecules with similar chemical structure.⁴⁰ This long acting inhibitor improves glucose tolerance, and islet function in mice.⁴⁰ Phase II clinical trials results released last summer were replaced NVP-DPP-728 by the new short acting inhibitor LAF-237.⁴¹ Interestingly, both DPP-IV inhibitors suppress glucagon release from liver with no effect on insulin secretion.⁴¹ The LAF-237 metformin combination

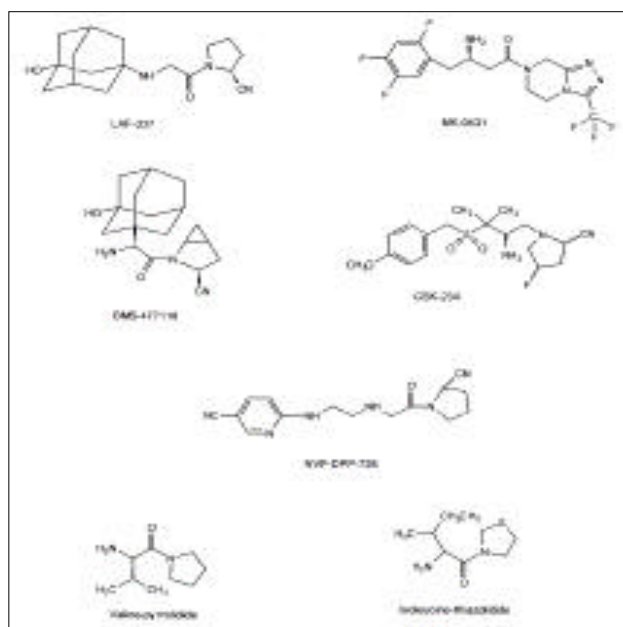


Figure 1 - Structures of dipeptidyl peptidase-IV inhibitors.

was useful in controlling blood glucose in type-2 diabetes not responding to metformin monotherapy.^{42,43} The LAF-237 is now in phase III trials and expected to be approved by 2006 in the USA. Isoleucine thiazolidine (P32/98), and FE-999011 cause sustained improvement in glucose tolerance, insulin sensitivity, and increase in β -cells proliferation with minimal side effects in Zucker rats (animals with insulin-resistant diabetes).^{44,45} The MK-0431 is in phase III trials with promising results. It controls blood glucose by increasing both intact GLP-1 and insulin secretion in type-2 diabetics.⁴⁶ The GSK-23A drug is in phase I trials while P-32/98 and BMS-477188 are in phase II trials. All of which have improved glucose level (hemoglobin A1c decrease by 1.1%), β -cell mass with limited side effects.⁴⁷

In conclusion, the GLP-1 analogues and DPP-IV have promising clinical results and may replace the current conventional therapy: insulin injection, metformin, and sulfonylurea in type-2 diabetics whose represent more than 90% of diabetic patients. These drugs may not only manage the disease but also could treat it in terms of long run administration by improvement of pancreatic functions.

References

- Bell GI, Santerre RF, Mullenbach GT, Hamster preproglucagon contains the sequence of glucagon and two related peptides. *Nature* 1983; 302: 716-718.
- Bell GI, Sanchez-Pescador R, Laybourn PJ, Najarian RC. Exon duplication and divergence in human preproglucagon gene. *Nature* 1983; 304: 368-371.
- Heinrich G, Gros P, Lund PK, Bently RC, Habener JF, Heinrich G. Preproglucagon messenger ribonucleic acid: nucleotide and encoded amino acid sequences of the rat pancreatic complementary deoxyribonucleic acid. *Endocrinology* 1984; 115: 2176-2181.
- Orskov C. Glucagon-like peptide-1, a new hormone of the enteroinsular axis. *Diabetologia* 1992; 35: 701-711.
- Kieffer TJ, Habener JL. The glucagon-like peptide. *Endocr Rev* 1999; 20: 876-913.
- Orskov C, Holst JJ, Nielsen OV. Effect of truncated glucagon-like peptide-1 [proglucagon-(78-107) amide] on endocrine section from pig pancreas, antrum, and nonantral stomach. *Endocrinology* 1988; 123: 2009-2013.
- Elliott RM, Morgan LM, Tredger JA, Deacon S, Wright J, Marks V. Glucagon-like peptide-1-(7-36)-amide and glucose-dependent insulinotropic polypeptide secretion in response to nutrient ingestion in man: acute post-prandial and 24-h secretion patterns. *J Endocrinol* 1993; 138: 159-166.
- 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 (INS-1)-cells. *Diabetologia* 1999; 42: 856-864.
- Stoffers DA, Kieffer TJ, Hussain MA, Drucker DJ, Egan JM, Bonner-Weir S. Insulinotropic glucagon-like peptide-1 agonists stimulate expression of homeodomain protein IDX-1 and increase β -cell mass in mouse pancreas. *Diabetes* 2000; 49: 741-748.
- Buteau J, El-Assaad W, Rhodes CJ, Rosenberg L, Joly E, Prentki M. Glucagon-like peptide-1 prevents β -cell glucolipototoxicity. *Diabetologia* 2004; 47: 806-815.
- 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.
- Flint A, Raben A, Ersboll AK, Holst JJ, Astrup A. The effect of physiological levels of glucagon-like peptide-1 on appetite, gastric emptying, energy and substrate metabolism in obesity. *Int J Obes Relat Metab Disord* 2001; 25: 781-792.
- Zander M, Madsbad S, Madsent JL. Effect of 6-weeks course of glucagon-like peptide-1 on glycaemia control, insulin sensitive, and β -cell function in type 2 diabetes: a parallel-group study. *Lancet* 2002; 359: 824-830.
- Nikolaidis LA, Mankad S, Sokos GG, Miske G, Shah A, Elahi D, et al. Effect of glucagon-like peptide-1 (GLP-1) can directly protect the heart against ischaemia/reperfusion injury. *Circulation* 2004; 109: 962-965.
- Yamamoto H, Lee CE, Marcus JN, Williams TD, Overton JM, Lopez ME, et al. Glucagon-like peptide-1 receptor stimulation increase blood pressure and heart rate and activates autonomic regulatory neurons. *J Clin Invest* 2002; 110: 43-52.
- Holst JJ, Gromada J. Role of incretin hormones in the regulation of insulin secretion in diabetic and nondiabetic human. *Am J Physiol Endocrinol Metab* 2004; 287: E199-E206.
- Nauck MA, Meier JJ. Glucagon-like peptide 1 and its derivatives in the treatment of diabetes. *Regul Pept* 2005; 128: 135-148.
- Visboll 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.
- Hansen L, Deacon CF, Orskov C, Holst JJ. Glucagon-like peptide-1-(7-36) amide is transformed to Glucagon-like peptide-1-(9-36) amide by dipeptidyl peptidase IV in the capillaries supplying the L cells of the porcine intestine. *Endocrinology* 1999; 140: 5356-5363.
- 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.
- Eng J, Kleinman WA, Singh L, Singh GR, Raufman JP. Isolation and characterization of exendin-4, an exendin-3 analogue, from Heloderma suspectum venom. Further evidence for an exendin receptor on dispersed acini from guinea pig pancreas. *J Biol Chem* 2003; 267: 402-405.
- Fineman MS, Bicsak TA, Shen LZ, Taylor K, Gaines E, Varns A. Effect on glycemic control of exenatide (synthetic exendin-4) additive to existing metformin and/or sulfonylurea treatment in patient with type 2 diabetes. *Diabetes Care* 2003; 26: 2370-2377.
- 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-E161.
- Holst JJ, Deacon CF. Glucagon-like peptide 1 and inhibitors of dipeptidyl peptidase IV in the treatment of type 2 diabetes mellitus. *Curr Opin Pharmacol* 2004; 4: 589-596.
- Dalton LW. Drugs for diabetes: new therapies targeting hormone in the gut promise more benefits than existing drugs and show potential for regenerating beta cells. *Chem Eng* 2004; 25: 59-65.
- Ribel U, Larsen MO, Rolin B, Carr RD, Wiken 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: 217-225.

27. Kim JG, Baggio LL, Bridon DP, Castaigne JP, Robitaille MF, Jette L, et al. Development and characterization of glucagon-like peptide 1-albumin conjugate: the ability to activate the glucagon-like peptide 1 receptor in vivo. *Diabetes* 2003; 52: 751-759.
28. Bloom M, Bock J, Duttaroy A, Grzegorzewski K, Moor P, Ou Y, et al. Albugon fusion protein: a long acting analogue of GLP-1 that provides lasting antidiabetic effect in animal. *Diabetes* 2003; 52: A112.
29. Liu HK, Green BD, Gault VA, McCluskey JT, McClenaghan NH, O'Harte FPM, et al. N-acetyl-GLP-1: a DPP IV-resistant analogue of glucagon-like peptide-1 (GLP-1) with improved effects on pancreatic α -cell associated gene expression. *Cell Biol Int* 2004; 28: 69-73.
30. O'Harte FPM, Mooney MH, Lawlor A, Flatt PR. N-terminally modified glucagon-like peptide-1 (7-36) amide exhibits resistance to enzymatic degradation while maintaining its antihyperglycaemic activity in vivo. *Biochem Biophys Acta* 2000; 1474: 13-22.
31. Juhl CB, Hollingdal M, Sturis J, Jakobsen G, Agero 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: 424-429.
32. Madsbad S, Schmitz O, Ransstam 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.
33. Nauck MA, Hompesch M, Filipczak R, Le TDT, Nielsen LL, Zdravkovic M. Liraglutide significantly improves glycemic control and reduced body weight compared with glimepiride as add-on to metformin in type 2 diabetes. *Diabetes* 2004; 53: A83.
34. Saad M, An B, Santiago O. The effect of NN2211, a long-acting GLP-1 derivative, on glycemic control and body weight in obese patients with type 2 diabetes. *Diabetologia* 2002; 45: A44.
35. Lawrence B, Dreyfus J, Wen S, Guicarc'h P, Drucker D, Castaigne JP. CJC-1131, a long acting GLP-1 derivative, exhibits an extended pharmacokinetic profile in healthy human volunteers. *Diabetes* 2003; 52: A125.
36. Deacon CF, Johnsen AH, Holst JJ. Degradation of glucagon-like peptide-1 by human plasma in vivo yields an N-terminally truncated peptide which is a major endogenous metabolite in vivo. *J Clin Endocrinol* 1995; 80: 952-957.
37. Rolin B, Deacon CF, Carr RD, Ahrén B. The major glucagon-like peptide-1 metabolites, GLP-1-(9-36)-amide, does not affect glucose or insulin levels in mice. *Eur J Pharmacol* 2004; 494: 283-288.
38. 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.
39. Weber AE, Kim D, Beconi M. MK-0431 is a potent, selective dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. *Diabetes* 2004; 53: A151.
40. 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
41. Ahren B, Landin-Olsson M, Jansson PA, Svensson M, Holmes D, Schweizer A. Inhibition of dipeptidyl peptidase-4 reduces glycemia, sustains insulin levels, and reduce glucagon levels in type 2 diabetes. *J Clin Endocrinol Metab* 2004; 89: 2078-2084.
42. Ahren B, Gomis R, Standl E, Mills D, Schweizer A, editors. A prolonged efficacy of LAF237 in patients with type 2 diabetes (T2DM) inadequately treated with metformin. 64th Scientific Sessions of American Diabetes Association; 2004 June 4-8; Orlando, USA. Orlando: American Diabetes Association; 2004.
43. Hinke SA, Kuhn-Wache K, Hoffmann T, Pederson RA, McIntosh CHS, Demuth HU. Metformin effects on dipeptidyl peptidase IV degradation of glucagon-like peptide-1. *Biochem Biophys Res Commun* 2002; 291: 1302-1308.
44. Pospisilik JA, Stafford SG, Demuth HU, Brownsey R, Parkhouse W, Finegood DT, et al. A 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; 52: 943-950.
45. 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: 1461-1469.
46. Herman GA, Zhao PL, Dietrich B. The DP-IV inhibitor MK-0431 enhances active GLP-1 and reduces glucose following an OGTT in type 2 diabetics. *Diabetes* 2004; 53: A82.
47. Heins J, Glund K, Hoffmann T, Metzner J, Demuth HU. The DP-IV inhibitor P93/01 improves glucose tolerance in humans with HbA1c greater than 6. *Diabetes* 2004; 53: A128.