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## Role of DPP IV Inhibitor in Type-2 Diabetes Mellitus

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### ABSTRACT

Incretin hormones are defined as intestinal hormones released in response to nutrient ingestion, which potentiate the glucose-induced insulin response. In humans, the incretin effect is mainly caused by two peptide hormones, glucose-dependent insulin releasing polypeptide (GIP), and glucagon-like peptide-1 (GLP-1). GIP is secreted by K cells from the upper small intestine while GLP-1 is mainly produced in the enteroendocrine L cells located in the distal intestine. Their effect is mediated through their binding with specific receptors, though part of their biological action may also involve neural modulation. GIP and GLP-1 are both rapidly degraded into inactive metabolites by the enzyme dipeptidyl-peptidase-IV (DPP-IV). In addition to its effects on insulin secretion, GLP-1 exerts other significant actions, including stimulation of insulin biosynthesis, inhibition of glucagon secretion, inhibition of gastric emptying and acid secretion, reduction of food intake, and trophic effects on the pancreas. As the insulinotropic action of GLP-1 is preserved in type 2 diabetic patients, this peptide was likely to be developed as a therapeutic agent for this disease.

**Key words:** Incretin hormones; Glucagon-like peptide-1; Insulin; Type 2 diabetes.

## INTRODUCTION

Incretins are a group of gastrointestinal hormones that cause an increase in the amount of insulin released from the beta cells of the islets of Langerhans after eating, even before blood glucose levels become elevated. They also slow the rate of absorption of nutrients into the blood stream by reducing gastric emptying and may directly reduce food intake. As expected, they also inhibit glucagon release from the alpha cells of the Islets of Langerhans [1, 2].

Because orally ingested glucose leads to a far greater insulin response than intravenous glucose with similar postprandial plasma glucose excursions, the phenomenon has been termed the "incretin effect". Up to two thirds of insulin normally secreted in relation to meal intake is thought to be due to the insulinotropic actions of the so called incretin hormones. Studies have revealed that secretion of incretin hormones is diminished in type 2 diabetes.

The incretin effect was finally defined as the phenomenon of oral glucose eliciting a greater insulin response than intravenous glucose infusions, irrespective of the same amount of glucose is infused or an equivalent rise in glycaemia is produced by the parenteral route. The incretin effect account for approximately 50 to 70% of the total insulin secreted after glucose ingestion.

### Historical Prospective:

The concept that factors secreted from the gut participate to the regulation of endocrine secretion was raised as early as the beginning of the 20th century. The term "secretin" was first used to define factors regulating pancreas secretion [3]. Later, the term "incretin" was introduced in the 1920's to describe these potential mediators. The connection between the gastrointestinal tract and the endocrine pancreas was confirmed in the 1960s, when insulin became measurable in plasma. Clinical studies showed that for an oral and an intravenous load of glucose producing identical increases in plasma glucose levels, the insulin secretory response was greater when glucose was administered orally. These findings suggested that not only glucose interacted with beta cells in the islets of Langerhans, but also gut factors were released, that stimulated insulin secretion.

### Two Major Incretin Hormones:

In humans, two peptide hormones have been identified as being responsible for the incretin effect, namely glucose-dependent insulin releasing polypeptide, GIP (formerly called gastric inhibitory polypeptide) and glucagon-like peptide-1, GLP-1. GIP and GLP-1 are both secreted in response to food ingestion and both potentiate the glucose-induced insulin response. Both are potent insulinotropic hormones released by oral glucose as well as ingestion of mixed meals.

### GIP

GIP was the first incretin to be described. GIP is a peptide of 42 amino acids belonging to the glucagon-secretin family of peptides, the members of which have pronounced sequence homology, particularly in the NH<sub>2</sub>-terminus. It is derived from a 153-amino acid precursor, but specific functions for other fragments of the precursor have not been identified. The GIP receptor has been cloned and is related to the receptors for the other members of the glucagon-secretin family. It is expressed in the islets and also in the gut, adipose tissue, heart, pituitary, adrenal cortex, and several regions of the brain.

GIP is secreted from specific endocrine cells, so-called K cells, with highest density in the duodenum but found in the entire small intestinal mucosa. Secretion is stimulated by absorbable carbohydrates and by lipids. GIP secretion is therefore greatly increased in response to meals, resulting in 10- to 20-fold elevations of the plasma concentration. Interaction of GIP with its receptor on the  $\beta$ -cells causes elevation of cAMP levels, which in turn increases the intracellular calcium concentration and enhances exocytosis of insulin-containing granules by a mechanism distal to the elevation of calcium.

## GLP-1

This was the second incretin to be discovered. GLP-1 is a product of the glucagon gene. It is expressed not only in pancreatic  $\alpha$ -cells but also in the L-cells of the intestinal mucosa, one of the most abundant endocrine cells of the gut. Here the primary translation product proglucagon is not cleaved to produce glucagon like in the islets but to release from its COOH-terminal part the two glucagon-like peptides GLP-1 and GLP-2, both showing 50% sequence homology with glucagon. GLP-1 secretion is stimulated by the presence of nutrients in the lumen of the gut, and its secretion throughout the day is highly correlated to the release of insulin.

GLP-1 is one of the most potent insulin-releasing substances known, exceeding that of GIP. Like GIP it interacts with a G protein-coupled receptor on the  $\beta$ -cells, which causes accumulation of cAMP, and most if not all of the subsequent effects seem to be secondary to this.

## GIP Action: Insights from Preclinical and Human Studies

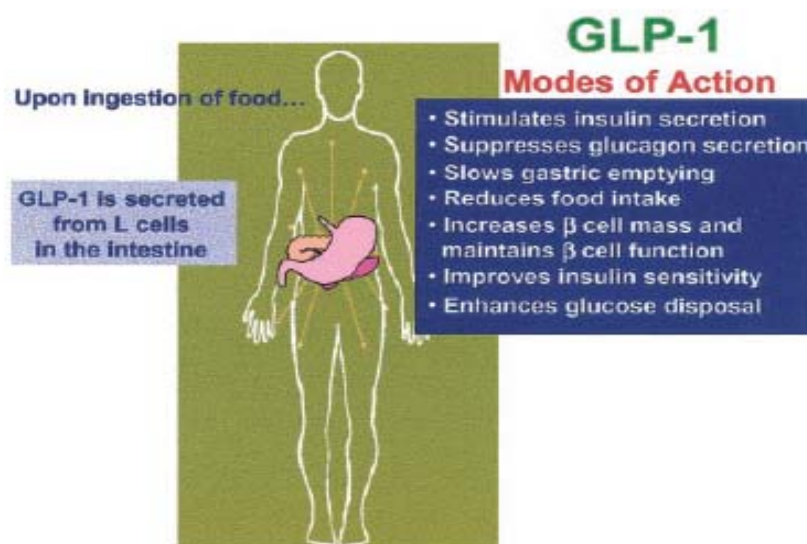
GIP was originally observed to inhibit gastric acid secretion (gastric inhibitory polypeptide), predominantly at supraphysiological dosages. Subsequent studies have demonstrated potent glucose-dependent insulin stimulatory effects from GIP administration in dogs and rodents. GIP also regulates fat metabolism in adipocytes, including stimulation of lipoprotein lipase activity, fatty acid incorporation, and fatty acid synthesis. Unlike GLP-1, GIP does not inhibit glucagon secretion or gastric emptying. GIP does promote  $\beta$ -cell proliferation and cell survival in islet cell line studies; whether GIP also induces  $\beta$ -cell growth or survival in diabetic rodents remains unclear.

The physiological actions of GIP have been deduced using GIP peptide antagonists, GIP receptor antisera, and GIP receptor knockout mice.  $\text{NH}_2$ -terminally truncated or modified GIP peptides such as GIP amide, GIP amide, or (Pro3)GIP block GIP binding to the GIP receptor with varying effectiveness, and attenuate the insulinotropic effects of exogenous GIP in vitro and endogenous GIP in vivo. Similarly, immunopurified antisera against the extracellular domain of the GIP receptor block GIP binding and attenuate glucose-dependent insulin secretion after oral glucose loading in rats and mice. Complementary evidence for the incretin-like actions of GIP is derived from analysis of GIP receptor null mice, which exhibit mild glucose intolerance after oral glucose loading.

## GLP-1 Preclinical Studies and Physiological Actions:

Original observations elucidating a role for GLP-1 in the potentiation of glucose dependent insulin secretion and insulin gene expression were followed by experiments demonstrating that GLP-1 also inhibits glucagon secretion and gastric emptying (Fig.2). Acute intracerebroventricular injection of GLP-1 or GLP-1 receptor (GLP-1R) agonists produces transient reduction in food intake, whereas more prolonged intracerebroventricular or peripheral GLP-1R agonist administration is associated with weight loss in some, but not all studies. GLP-1 actions on food intake appear related in part to overlapping actions on central nervous system aversive signaling pathways, which remain a topic of intense interest. In contrast to GIP, the spectrum of actions delineated for GLP-1 that promote glucose lowering (regulation of insulin and glucagon secretion, inhibition of gastric emptying, and reduction of food intake) appear comparable in diabetic versus non-diabetic animals of various ages.

GLP-1 exerts actions on  $\beta$ -cells independent of acute stimulation of insulin secretion. Incubation of isolated rat islet cells with GLP-1 recruited nonresponsive glucose-resistant  $\beta$ -cells to a functional state of glucose-responsive insulin secretion, designated glucose competence. GLP-1R agonists also promote insulin biosynthesis,  $\beta$ -cell proliferation, and survival, and stimulate differentiation of exocrine cells or islet precursors toward a more differentiated  $\beta$ -cell phenotype. The GLP-1R-dependent augmentation of  $\beta$ -cell mass has been demonstrated in diverse experimental models, including neonatal rats administered streptozotocin and exendin-4 and normal Wistar rats ages 6 and 22 months infused with native GLP-1 for 5 days. Similarly, GLP-1R agonists promote  $\beta$ -cell proliferation and expansion of functional islet mass after partial pancreatectomy in rats aged 4–5 weeks or in neonatal rat pups subjected to experimental intrauterine growth retardation. The expansion of  $\beta$ -cell mass after GLP-1R agonist administration prevents or delays the occurrence of diabetes in *db/db* mice and GK diabetes-prone rats. Furthermore, the induction of islet proliferation after GLP-1R activation has been seen with a broad range of GLP-1R agonists, including native GLP-1, exendin-4, NN2211, and CJC-1131.



**Fig. 1: The major biological actions of GLP-1**

The physiological importance of GLP-1 action has been studied using GLP-1R antagonists. Infusion of the peptide exendin into rats, mice, baboons, and humans produces an increase in fasting glucose and glycemic excursion after oral glucose loading in association with reduced levels of circulating insulin. Exendin also produces abnormal glycemic excursion after nonenteral glucose loading in mice. These findings illustrate that transient disruption of GLP-1 action consistently perturbs the incretin and nonincretin actions of GLP-1 on gluoregulation. Acute intracerebroventricular injection of exendin increases food intake in satiated rats, whereas repeated daily intracerebroventricular administration of exendin increases food intake and weight gain. Similarly, acute exendin administration increases gastric emptying after glucose ingestion in fistulized rats. Comparable studies with exendin in humans have demonstrated the essential role of GLP-1 action for glucose control via regulation of glucagon and insulin secretion. Hence, the majority of actions observed after exogenous administration of GLP-1R agonists are also physiologically essential, as revealed by acute interruption of GLP-1 action.

**Table 1. Properties and Biological actions of GIP and GLP-1**

GIP	GLP-1
42-Amino acid peptide	30/31-Amino acid peptide
Released from duodenum	Released from distal small bowel and colon
NH <sub>2</sub> - terminal inactivation by DPP-IV	NH <sub>2</sub> - terminal inactivation by DPP-IV
Stimulates insulin secretion	Stimulates insulin secretion
Minimal effect on gastric emptying	Inhibits gastric emptying
No effect on glucagon secretion	Inhibits glucagon secretion
No regulation of satiety and body weight	Inhibits food intake and body weight
Promotes expansion of $\beta$ -cell mass	Promotes expansion of $\beta$ -cell mass
Normal GIP secretion in diabetic subjects	Reduced GLP-1 secretion in diabetic subjects
defective GIP response in type 2 diabetes	Preserved GLP-1 response in type 2 diabetes

### **Incretin Synthesis, Pharmacokinetic and Effectors:**

GIP and GLP-1 are both members of the glucagon peptide superfamily, sharing a close amino acid homology. GIP is a single 42 amino acid peptide derived from the processing of a 153 amino acid precursor, whose 10 kilo base-spanning gene is located on chromosome 17 in humans. GIP is secreted in a single bioactive form by K cells and released from the upper small intestine (duodenum and proximal jejunum), in response to the oral ingestion of carb-

ohydrates and lipids. GLP-1 is a product of the proglucagon gene, spanning 10 kilobases and located on the long arm of chromosome 2, that encodes not only GLP-1 but also glucagon, GLP-2 and other proglucagon-derived peptides. Glucagon is the main product of post transcriptional processing of proglucagon in the endocrine pancreas. GLP-1 is produced together with GLP-2 and glicentin (enteroglucagon) as the main products in the enteroendocrine L cells. Despite close structural homology, GLP-2 does not share the same biological action as GLP-1, but rather acts as a regulator of growth in the intestinal tract.

GLP-1 is mainly expressed in mucosal L cells located predominantly in the distal intestine (ileum and colon), and is also expressed in pancreatic alpha cells, as well as in neurons from several brain areas (hypothalamus, pituitary, nucleus of the tractus solitarius, reticular nucleus). GLP-1 is secreted from L cells in two bioactive forms, GLP-1 and the predominant circulating active form GLP-1 amide, also called “truncated” GLP-1. Both peptides are equipotent, with a same plasma half-life and identical activity through the same receptor.

Despite the distal location of L-cells in the gastrointestinal tract, GLP-1 is released into the circulation within minutes following oral ingestion of nutrients, suggesting that this prompt release is more indirectly controlled by neural and endocrine factors initiated by nutrient entry in the proximal gastrointestinal tract, rather than directly stimulated by contact of L-cells with nutrients. Although these factors remain largely unknown in humans, experimental studies in rodents suggest that the vagal nerve, through muscarinic receptors M3, is a contributing factor. However, L cells do exist in the proximal intestine. Recently, the sweet taste receptor subunit T1R3 and the taste G protein gustducin of the tongue have been shown to be also expressed in enteroendocrine cells and to modulate glucose transport in the enterocytes (via the sodium-dependent glucose transporter SGLT1). Animal models lacking gustducin display defective GLP-1 secretion in response to glucose. *In vitro*, GLP-1 release from a human L cell line, is promoted by sugars and the noncaloric sweetener sucralose, and blocked by the sweet receptor antagonist lactisole or siRNA for alpha-gustducin. Therefore, taste receptors participate in the GLP-1 release in response to glucose.

### Potential effects of GLP-1 in type 2 diabetes

Michael A. Nauck (bad Laukenberg em Nacht, Germany) discussed GLP-1 and glucose-dependent insulinotropic peptide (GIP) in type 2 diabetes. GLP-1 is produced by L-cells in the distal small bowel, while GIP comes from K-cells in proximal small intestine. Classically, the incretin effect has been demonstrated by comparing effects of intravenous versus oral glucose administered to produce similar plasma glucose curves and hence “glycemic stimulus.” Under such conditions, oral glucose produces a threefold higher insulin and C-peptide response. In persons with type 2 diabetes, however, the incretin effect is attenuated or even completely lost. One explanation would be that the relative secretion of hormones mediating the incretin effect is decreased. Indeed, comparing persons with and without type 2 diabetes, differences in GLP-1 appear ~1 h after oral glucose, while GIP may either be hypo- or hypersecreted and therefore was not felt to explain the lack of incretin effect in type 2 diabetes. Studies comparing the effects of GIP and GLP-1 in diabetic and nondiabetic persons show, however, that the response to GIP is decreased while that to GLP-1 is similar in diabetic and nondiabetic persons, suggesting that the lack of GIP effect may indeed be important in type 2 diabetes. Comparing bolus injections of GLP-1 and GIP, persons with type 2 diabetes do show response to GIP as to GLP-1, but longer periods of infusion of GIP fail to increase insulin levels in the fashion seen with GLP-1, implying that the mechanism is complex. In a study of GIP infusion during hyperglycemia, approximately half of a group of relatives of persons with type 2 diabetes had attenuation of insulin response. Although this might seem a likely candidate as an early marker of type 2 diabetes, there was no difference in glucose tolerance between responders and nonresponders either at baseline or at 4-year follow-up, further indicating the intricacy of these effects.

### GIP/GLP-1 Receptor Knockout Studies

The quantitative impact of incretin hormones can be derived from studies of animals with a targeted disruption of the GIP and GLP-1 receptor genes. In both cases, oral glucose tolerance is diminished. The disruption of signaling through a single incretin receptor may not fully disclose the importance of that particular pathway, because compensatory mechanisms may be active. For example, GLP-1 receptor knockout mice display enhanced secretion of and sensitivity to GI. Nevertheless, double incretin receptor knockout mice, which lack the possibility for compensation through any known important incretin hormone, do not present with an overtly diabetic phenotype.

Therefore, it is unlikely that impairments in incretin secretion and insulinotropic action alone explain the phenotype of type 2 diabetes.

### **Inactivation of incretin hormones**

GIP and GLP-1 are rapidly degraded by the enzyme dipeptidyl peptidase-4 (DPP-4).<sup>34</sup> DPP-4 cleaves the active peptide at position 2 alanine (N-terminal) resulting in inactive peptide.<sup>41</sup> DPP-4 is widely expressed in human tissues including the brain, lungs, kidneys, adrenals, pancreas, intestine and lymphocytes, among others.<sup>41</sup> Interestingly, it is found in the endothelial cells of the blood vessels that drain the intestinal mucosa, where the L-cells are situated. This suggests that the majority of GLP-1 is inactivated almost immediately following secretion. This rapid inactivation of GLP-1 and GIP contributes to a half-life of <2 minutes and 5–7 minutes respectively.

### **Incretin mimetics for type 2 diabetes:**

#### **DPP-4**

Circulating levels of DPP-4 activity have been reported to be higher in some studies of subjects with chronic hyperglycemia and type 2 diabetes; however, whether circulating DPP-4 activity correlates with the levels of active plasma GLP-1 in individual human subjects is not known. The observation that DPP-4 was capable of cleaving the incretin peptides GIP and GLP-1 in human serum in vitro, together with the demonstration that chemical inhibitors of DPP-4 prevented the degradation of GIP and GLP-1, firmly established the importance of DPP-4 as a critical determinant of incretin inactivation. Subsequent studies demonstrated reduced cleavage of intact GLP-1 amide and GIP in serum from DPP-4-deficient rats in vitro or following infusion of the peptides into DPP-4-deficient rats in vivo, providing complementary evidence for the importance of DPP-4 in the control of incretin inactivation. Moreover, both GLP-1 amide and the NH<sub>2</sub>-terminal DPP-4-generated metabolite GLP-1 amide were identified in plasma from both fasted and fed humans, and inhibitors of DPP-4 prevented the conversion of GLP-1 amide to GLP-1 amide in human plasma in vitro. Similarly, the majority of circulating immunoreactive GIP in human plasma is the NH<sub>2</sub>-terminally cleaved GIP peptide, accounting for ~70% of total plasma GIP immunoreactivity in the fasting state and 58% of total GIP after meal ingestion. Furthermore, exogenous administration of either GIP or GLP-1 via the subcutaneous or intravenous routes was associated with the rapid degradation of both peptides within minutes to the DPP-4 metabolites GIP and GLP-1 amide, respectively. Hence, DPP-4 is a principal determinant of the circulating *t*<sub>1/2</sub> of intact bioactive GIP and GLP-1.

### **Studies in rats and mice with inactivating DPP-4 mutations**

The biological importance of DPP-4 has been examined in rats with a naturally occurring loss of function mutation in the DPP-4 gene and in mice with targeted genetic inactivation of DPP-4. A strain of Fischer 344 (F344) rats originally identified in Japan harbor a Gly633-Arg mutation in the DPP-4 gene within the active site of the enzyme. The mutant DPP-4 protein is synthesized appropriately, yet it is not exported out of the endoplasmic reticulum and is rapidly degraded without being processed to the mature active enzyme. Subsequent studies identified heterogeneity in baseline levels of DPP-4 activity in different inbred rat strains, emphasizing the importance of careful characterization of enzymatic activity in different rodent models. F344 rats exhibit improved glucose tolerance and increased levels of plasma GLP-1 and insulin following oral glucose challenge. Furthermore, high-fat feeding of F344 rats for 7 weeks was associated with reduced weight gain, increased levels of intact GLP-1, improved glucose tolerance, and enhanced insulin sensitivity as assessed by homeostatic model assessment. Hence, loss of DPP-4 activity in rats is associated with potentiation of endogenous GLP-1 action and improvement of glucose tolerance.

**Different Approaches to Diabetes Therapy**— Although GLP-1 receptor agonists (“incretin mimetics”) and DPP-4 inhibitors (“incretin enhancers”) are based on antidiabetic properties of insulinotropic gut hormones (“incretins”), they represent different approaches to the therapy of type 2 diabetes.

### **Similarities between GLP-1 and DPP-4 inhibitors**

Treatment with GLP-1 receptor agonists (“incretin mimetics”) and DPP-4 inhibitors (“incretin enhancers”) are based on antidiabetic properties of insulinotropic gut hormones (“incretins”). Although they represent different approaches to therapy in patients with type 2 diabetes, there are notable similarities (Table 1).

First, both therapy approaches engender significant and clinically relevant improvement in glycemic control regarding fasting plasma glucose, postprandial glucose, and A1C. Both treatment modalities benefit from the glucose-dependent effect of GLP-1 on insulin secretion and glucagons inhibition, whereby improvements in glucose control can be achieved with minimal risk of hypoglycemia when combined with metformin or thiazolidinedione (TZD). However, when GLP-1 is combined with sulfonylureas, the risk of hypoglycemia appears to be similar to that of sulfonylureas alone.

### Differences between GLP-1 and DPP-4 inhibitors

Incretin mimetics as peptides have to be injected subcutaneously. They raise the concentrations of GLP-1 receptor agonists to pharmacologically high levels, leading to concentrations 6- to 10-fold that of the physiological ones found in the postprandial state. The exogenous administration of GLP-1 receptor agonists with a long biological half-life results in constantly high plasma concentrations and consecutively in a continuous and exclusive stimulation of the GLP-1 receptor. This stimulatory route with high GLP-1 agonist plasma concentration is thought to mediate the predominantly endocrine and systemic effects of GLP-1. Apart from the insulinotropic and glucagonostatic actions, the actions of incretin mimetics include the slowing of gastric emptying that may result in sensations of fullness, or nausea at initiation of therapy, as well as a stimulation of satiety in the central nervous system. Currently, it is not known to what exact extent both effects contribute to weight loss and reduced appetite. In clinical studies, patients receiving incretin mimetics had body weight loss irrespective of nausea as an adverse event, favoring a regulatory action on the central nervous system, whereas patients treated with DPP-4 inhibitors did not substantially change their weight.

**Advantages of Incretin Mimetics and DPP-4 Inhibitors**— Based on currently available information (phase 3 studies designed for drug approval), incretin mimetics and DPP-4 inhibitors have advantages over other antidiabetic drugs.

### History of Dipeptidyl Peptidase IV (DPP-IV)

Since its discovery in 1967, serine protease DPP-4 has been a popular subject of research. Inhibitors of DPP-4 have long been sought as tools to elucidate the functional significance of the enzyme. The first inhibitors were characterized in the late 1980s and 1990s. Each inhibitor was important to establish an early structure activity relationship for subsequent investigation. It should be noted that the inhibitors fall into two main classes, those that interact covalent with DPP-4 and those that do not. DPP-4 is a dipeptidase that selectively binds substrates that contain proline at the P1-position, thus many DPP-4 inhibitors have 5-membered heterocyclic rings that mimic proline, e.g. pyrrolidine, cyanopyrrolidine, thiazolidine and cyanothiazolidine. These compounds commonly form covalent bonds to the catalytic residue Ser630. In 1994, researchers from Zeria Pharmaceuticals unveiled cyanopyrrolidines with a nitrile function group that was assumed to form an imidate with the catalytic serine. Concurrently other DPP-4 inhibitors without a nitrile group were published but they contained other serine-interacting motifs, e.g. boronic acids, phosphonates or diacyl hydroxylamines. These compounds were not as potent because of the similarity of DPP-4 and prolyl oligopeptidase and also suffered from chemical instability. Ferring Pharmaceuticals filed for patent on two cyanopyrrolidine DPP-4 inhibitors which they published in 1995. These compounds had excellent potency and improved chemical stability. In 1995, Edwin B. Villhauer at Novartis started to explore N-substituted glycinyln-cyanopyrrolidines based on the fact that DPP-4 identifies N-methylglycine as an N-terminal amino acid. This group of new cyanopyrrolidines became extremely popular field of research in the following years. Some trials with dual inhibitors of DPP-4 and vasopeptidase have been represented, since vasopeptidase inhibition is believed to enhance the antidiabetic effect of DPP-4 inhibition by stimulating insulin secretion. Vasopeptidase-inhibiting motif is connected to the DPP-4 inhibitor at the N-substituent.

### Structure of DPP-4

X-ray structures of DPP-4 give detailed information about the structural characteristics of the binding site. Many structurally diverse DPP-4 inhibitors have been discovered and it is not that surprising while considering the Properties of the binding site:

1. A deep lipophilic pocket combined with several exposed aromatic side chains for achieving high affinity small molecule binding.
2. A significant solvent access which makes it possible to tune the physico-chemical properties of the inhibitors that leads to better pharmacokinetic behavior

## Binding site

DPP-4 inhibitors usually have an electrophilic group that can interact with the hydroxyl of the catalytic serine in the active binding site. Frequently that group is a nitrile group but can also be boronic acid or diphenyl phosphonate. This electrophilic group can bind to the imidate complex with covalent bonds and slow, tight-binding kinetics but this group is also responsible for stability issues due to reactions with the free amino group of the P2-amino acid. Therefore inhibitors without the electrophilic group have also been developed, but these molecules have shown toxicity due to affinity to other dipeptidyl peptidases, e.g. DPP-2, DPP-8 and DPP-9. DPP-4 inhibitors span diverse structural types. In 2007 few of the most potent compounds contain a proline mimetic cyanopyrrolidine P1 group. This group enhances the potency, probably due to a transient covalent trapping of the nitrile group by the active site Ser630 hydroxyl, leading to delayed dissociation and slow tight binding of certain inhibitors. When these potency enhancements were achieved, some chemical stability issues were noted and more advanced molecules had to be made. To avoid these stability issues, the possibility to exclude the nitrile group was investigated. Amino acids with aryl or polar side chains did not show appreciable DPP-4 inhibition and in fact, all compounds without the nitrile group in this research suffered a 20 to 50-fold loss of potency corresponding to the compounds containing the nitrile group.

## Clinical Development of DPP-IV Inhibitors

DPP-IV exerts its biological effects via 2 distinct mechanisms of action. First, it binds ADA and, when activated, conveys intracellular signals independent of its enzymatic function via dimerization and activation of intracellular signaling pathways. Second, when functioning as an enzyme, DPP-IV rapidly inactivates the insulinotropic hormone GLP-1. Thus, inhibition of DPP-IV by DPP-IV inhibitors enhances the hormone activity of GLP-1 and other bioactive peptides (e.g., GIP, pituitary adenylyl cyclase activating polypeptide-38 [PACAP38] and gastrin-releasing peptide [GRP]), thereby stimulating the release of insulin and reducing the secretion of glucagon. Both effects contribute to regulation of elevated blood glucose levels in type 2 diabetic patients. The major advantage of DPP-IV inhibitors is its ability to achieve sustainable reductions in hemoglobin A1c with an orally administered, well-tolerated agent. In addition, available data from clinical phase II studies suggest that long-term treatment with DPP-IV inhibitors was not associated with weight gain [52-54]; however, this was not observed in animal studies.

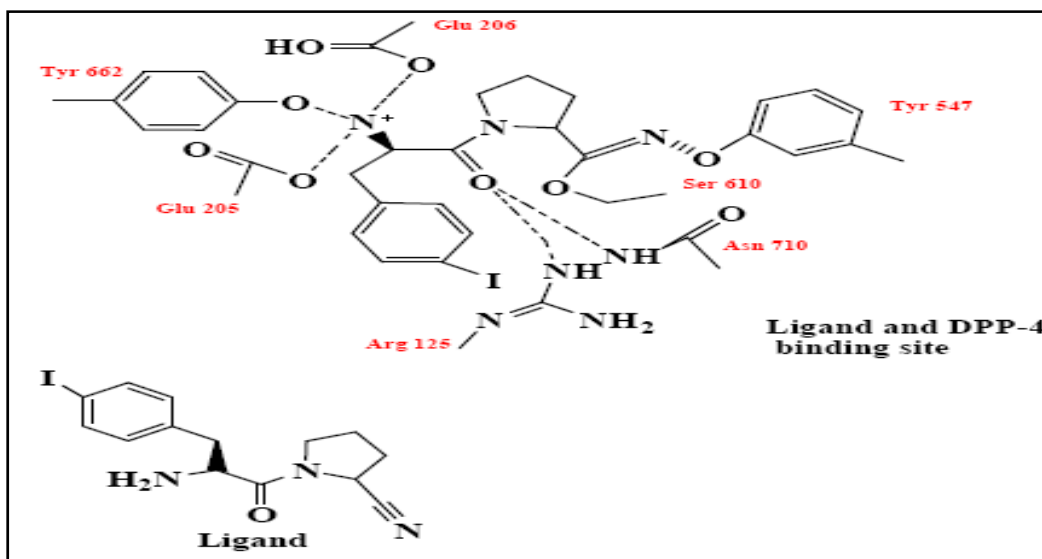


Fig.1. The key interaction between the ligand and DPP-4 complex. The ligand's basic amine forms a hydrogen bonding network. The nitrile reacts with the catalytic active serine and forms an imidate adduct.

## Discovery and Development of DPP-4 Inhibitors

Currently, worldwide sales of drugs for type 2 diabetes exceed \$12 billion (€9.9 billion) per year. The increased interest of the pharmaceutical industry in DPP-IV inhibitors reflects their market attractiveness and patent application.



There are over 100 patents filed with several different chemical structures, including amino acid amide, carbocyclic, alkylamine, and heterocyclic (mainly pyrrolidine, pyridine, and xanthine derivatives). With available favorable clinical and preclinical studies, DPP-IV inhibitors have become a research area of intense focus with a number of pharmaceutical companies involved in the development of what is hoped is a new diabetes therapy that will affect approximately 8-10% of the adult western population.

Although, a number of DPP-IV inhibitors have been described, all have limitations relating to potency, stability, or toxicity. Accordingly, a great need exists for novel DPP-IV inhibitors which are useful in treating conditions mediated by DPP-IV inhibition and which do not suffer from the abovementioned limitations.

**Table 2. Similarities of incretin based therapies**

Properties/action	Incretin mimetics	DPP-4 inhibitors
Glucose-dependent insulin secretion	Yes	Yes
Glucose-dependent glucagonostatic effect	Yes	Yes
Effect on fasting plasma glucose (reduction)	By 1.4-3.4 mmol/l	By 1.0-1.4 mmol/l
Effect on postprandial glucose	Yes	Yes (but weaker)
Effect on A1C (reduction)	By 0.8-1.8%	By 0.5-1.1%
Effect on (pro)insulin biosynthesis	Yes	Yes
Improved in-vivo $\beta$ -cell function (in humans)	Yes*	Yes*
Beneficial cardiovascular effects	Probable	Not proven

\*As determined when patient received treatment (lasting effects need to be proven after washing out treatment)

### DPP-IV inhibition – diabetes treatment

Glucagon-like peptide-1 (GLP-1) and gastric inhibitor peptide (GIP) are naturally occurring hormones (incretins) that are released from cells in the gut in response to food. They bind to receptors on pancreatic beta cells stimulating the release of the hormone insulin, responsible for the regulation of blood sugar levels. Treatment with vildagliptin does not appear associated with weight gain, which is an important benefit for patients with type 2 diabetes.

**Future Prospects:** It is widely held that the primary physiological role of incretins is to modulate plasma glucose. However, several independent lines of inquiry indicate that incretins have important functions relevant to pathophysiologies other than diabetes. There is a growing body of evidence indicating that incretin-related peptides have cardioprotective and neuroprotective actions that may combat other human diseases. At this stage it is too early to tell whether DPP-4 inhibitors could play a role in these actions. Their use will need to be reviewed periodically as and when new evidence comes to light.

### Cardio protective Effects

Patients with diabetes are characterised by an increased risk of developing both microvascular complications (e.g. retinopathy, nephropathy and neuropathy) and atherosclerotic macrovascular disease, potentially leading to the development of peripheral vascular disease, stroke and heart failure. In the UK, cardiovascular disease (CVD) is the leading cause of mortality and is linked to premature death in up to 40% of the population. Patients with diabetes are characterised by significantly elevated CVD risk compared with normoglycaemic individuals.

The Framingham Heart Study determined that heart failure was twice as common in men with diabetes and five times as common in women with diabetes who were 45–74 years of age compared with the normal population and that this association was even stronger in younger patients. Recent evidence suggests that in addition to its established glucose lowering actions, GLP-1 may also exert several beneficial actions on the cardiovascular system, including improved left ventricular function and improved recovery after myocardial ischaemia. This introduces new therapeutic possibilities for the use of GLP-1 compounds in the treatment of CVD in both normal patients and those with diabetes. Recent evidence supports the existence of GLP-1 signalling pathways independent of the classic GLP-1R. The existence of multiple GLP-1Rs within the cardiovascular system has not been ruled out. Several studies have suggested that the metabolically inactive form of GLP-1 (GLP-1(9–36) amide) may play a significant

cardiovascular role and this has potential implications for DPP-4 inhibitor therapies. The emerging cardiovascular actions of GLP-1 and the potential for GLP-1 as a treatment for CVD in both diabetic and non-diabetic patients has recently been reviewed in depth.

### Neuroprotective effect

The central effects of GLP-1 to inhibit feeding are widely appreciated. They appear to be mediated by GLP-1 receptors located in the arcuate nucleus and other hypothalamic regions. Indeed, GLP-1 synthesised in the brain may activate these receptors as well as acting peripherally. GLP-1 triggers satiety pathways by gaining direct access through the blood-brain barrier in addition to activating sensory vagal afferent nerve fibres and other networks comprising the gut-brain axis. Stable GLP-1 receptor mimetics promote clinically significant bodyweight loss, whereas DPP-4 inhibitors are weight-neutral and generally lack effects on gastric emptying, presumably due to much lower concentrations of endogenous GLP-1 being achieved following therapy. GIP also lacks effects on feeding, but it is increasingly clear that, like GLP-1, many brain regions synthesise GIP and are endowed with functional GIP receptors. Such observations fit well with growing evidence in preclinical studies that both GLP-1 and GIP increase cognition and exert potentially important central neuroprotective actions. Thus, DPP-4-resistant analogues of GLP-1 and GIP have recently been shown to improve hippocampal neurotransmitter release and synaptic plasticity, also protecting synapses from the detrimental neurodegenerative effects of beta-amyloid fragments. These observations suggest a possible therapeutic benefit of incretin hormones in Alzheimer's and other diseases associated with impaired cognitive function. Indeed GLP-1 receptor knockout mice demonstrate impaired synaptic plasticity and memory formation. Whether similar benefits might follow from the clinical use of DPP-4 inhibitors remains to be explored. Direct central effects can be discounted because DPP-4 inhibitors are generally designed not to cross the blood-brain barrier and the central nervous system contains very low levels of DPP-4 anyway. However, recent studies report a beneficial effect of sitagliptin on cognition and amyloid deposition in Alzheimer's prone mice. Neuroprotective effects of increased circulating incretin levels passing into the brain and/or by activation of peripheral sensory neural pathways are worthy of consideration, especially given their increasing recognition in glucoregulatory incretin effects.

### CONCLUSION

GIP and GLP-1 are both incretin hormones secreted in response to meal ingestion that potentiate the glucose-induced insulin response. In addition, GLP-1 plays an important role in inhibiting glucagon secretion. Trophic effect of GLP-1 on pancreatic beta-cells were also demonstrated in animal models. Other physiological actions of GLP-1 include the inhibition of GI secretion and gastric emptying, and the reduction of food intake. In contrast with GIP, the insulinotropic action of GLP-1 has been shown to be preserved in type 2 diabetic patients. All these findings support GLP-1 as an attractive alternative therapy to conventional antidiabetic agents. Many questions are still unanswered such as the causes of the incretin defect associated with type 2 diabetes. Nevertheless, the study of incretin hormones brought new concepts and fuelled food related integrated physiology.

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