Review
Of
Literature
2. Review of Literature

2.1. Diabetes

There are two major types of diabetes (1 and 2). Type 1 occurs in 5-10% of cases and is largely recognized as an autoimmune disease whereby the β-cells are destroyed by the body's own antibodies. Since insulin can no longer be produced, the only effective treatment is daily insulin injections. It is estimated that 90-95% of diabetes are type 2 and is usually caused by a combination of resistance to the effects of insulin, particularly in adipose and muscle tissue, but also in liver, and its decreased production by β-cells in the pancreas. Fasting plasma glucose (FPG) concentrations are normally strictly maintained within 90-120 mg/dl but in type 2 diabetes the person is unable to maintain glucose levels within this range. The disease is characterized by a FPG of > 126 mg/dl or by a two hour oral glucose tolerance test (OGTT) of > 200 mg/dl. The onset of diabetes is preceded by a pre-diabetic state with FPG between 110-125 mg/dl and is referred to as impaired fasting glucose (IFG). Alternatively, it is recognized by a two hour OGTT of 140-200 mg/dl and is referred to as impaired glucose tolerance (IGT). The American Diabetes Association (ADA) has recommended reducing the threshold for IFG to 100-125 mg/dl (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus., 2003). The WHO has chosen not to adopt this more stringent level because they believe there is no evidence to suggest any benefit in reducing adverse outcomes or progression to diabetes (WHO., 2006).

Glucose in the blood may bind to hemoglobin to give glycosylated hemoglobin (HbA1c). HbA1c is directly proportional to blood glucose levels and are perhaps the most accurate indicator because it reflects plasma glucose levels over the previous 2-3 months. This would appear to be the ideal standard for assessing glycemic control, however, the WHO does not consider HbA1c levels a suitable diagnostic test for diabetes or pre-diabetes for two reasons. First, this test is not readily available in many parts of the world. Secondly, HbA1c levels are influenced by other factors completely unrelated to diabetes such as anemia, pregnancy and uraemia. Because FPG and OGTT are the most applicable means of accessing glucose levels, the WHO has decided that these should remain the standard for diagnosis of diabetes (WHO., 2006).

2.2. Complications
The effects of unregulated glucose control can lead to severe macro and microvascular complications. In fact, the correlation of these complications with glucose levels have been used to derive the cut offs for diagnosis of diabetes mentioned above (WHO., 2006). Diabetes primarily affects the heart, blood vessels, eyes, kidney and nerves (World Health Organization Fact Sheet N° 312., 2007). It is a leading cause of blindness and renal failure. Microvascular complications refer to those affecting small blood vessels in the retina, kidney, and peripheral nerves and can lead to retinopathy, nephropathy and neuropathy, respectively. Diabetic retinopathy occurs as a result of long-term damage to blood vessels in the retina and can lead to blindness or severe visual impairment. Diabetes can also cause the development of cataract through the formation of sorbitol deposits on the lens of the eye. Sorbitol is a product of the polyol pathway formed by the action of aldose reductase, which becomes over expressed in type 2 diabetes, and is believed to be intimately involved with organ damage. Diabetes is among the leading causes of kidney failure and 10-20% of diabetics die from this (World Health Organization Fact Sheet N° 312., 2007). Diabetic neuropathy occurs as a result of damage to the nerves and results in tingling, pain, numbness and weakness in the extremities, which left untreated, can lead to infection, ulceration and possibly amputation. Macrovascular complications refer to diseases affecting large blood vessels in the heart, brain and peripheral circulation leading to cardiovascular diseases such as atherosclerosis, heart attack and stroke, which are responsible for 50% of deaths of diabetics (World Health Organization Fact Sheet N° 312., 2007).

It is hypothesized that there are four main mechanisms by which hyperglycemia induces microvascular and macrovascular complications (Brownlee., 2001); (1) increased polyol pathway flux (2) increased glycation end-product formation; (3) activation of protein kinase C and (4) increased hexosamine pathway flux. A common effect of each is that they increase the production of superoxide by the mitochondrial electron-transport chain. Superoxide is a reactive oxygen species that leads to oxidative stress and can subsequently cause the tissue damage that is observed in diabetes. This suggests that antioxidants, as free-radical scavengers, may be used therapeutically in the future to prevent the complications associated with diabetes (Brownlee., 2001).

2.3. Regulation of Glucose Homeostasis
Glucose is the major source of cellular energy and its concentrations are controlled by a number of hormones, the most important being insulin and glucagon. Insulin is secreted by β-cells when blood glucose concentration rises and reduces glucose levels by two general mechanisms; (1) inhibition of hepatic glucose production (glycogenolysis and gluconeogenesis) and (2) increasing glucose uptake into muscle and fat tissue. Glucagon is a hormone secreted by pancreatic α-cells in response to low concentrations of glucose and is responsible for elevating blood glucose levels. It acts principally at the liver and antagonizes the effects of insulin by increasing glycogenolysis and gluconeogenesis and also inhibiting glycogenesis and glycolysis. Other hormones also function in maintaining normal glucose levels. These include amylin, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). Amylin is actually secreted with insulin from β-cells and functions in decreasing gastric emptying, which limits glucose excursions following a meal. GLP-1 and GIP are incretins, or gut derived hormones, which have a multitude of effects, two of which are to promote the synthesis and secretion of insulin from β-cells. A decrease in the effect of these incretins contributes to the progression of diabetes.

2.4. Pathogenesis
The pathogenesis of type 2 diabetes is complex but typically begins with insulin resistance at target organs such as liver, muscle and adipose. In order to compensate for this, there is initially an increase in insulin production. This hyperinsulinemic state is only temporary and over time insulin secretion diminishes due to progressive β-cell deterioration. The combined effects of insulin resistance and β-cell dysfunction results in a diminished capacity to limit hepatic glucose production as well as to decrease uptake and utilization of glucose in muscle and adipose tissue (i.e. insulin resistance). Insulin resistance is a complex disease that typifies the metabolic syndrome and is the result of a number of defects along the insulin signaling cascade (Moneva & Dagogo-Jack.,2002). Other likely factors include increased concentrations of free-fatty acids (FFA's), tumor necrosis factor-α (TNF-α) and the hormone resistin (Moneva & Dagogo-Jack.,2002). Elevated FFA's produce insulin resistance by inhibiting glucose uptake and its oxidation (i.e. glycolysis) in skeletal muscle. FFA's also increase hepatic gluconeogenesis. Both TNF-α and resistin are produced by adipose tissue in greater
amounts in obese diabetic individuals. TNF-α impairs insulin action while resistin is known to antagonize the effects of insulin (Moneva & Dagogo-Jack., 2002).

Increased hepatic glucose production in type 2 diabetes is attributed to both hepatic insulin resistance and increased glucagon levels (Triplitt et al., 2006). β-cells can compensate for resistance by secreting more insulin. This hyperinsulinemic state is only temporary, as β-cells cannot maintain insulin levels required to maintain euglycemia. This is referred to as the "petering out" effect and occurs due to apoptosis of β-cells. High glucose and FFA's contribute to β-cell malfunction, in a condition called glucolipotoxicity. When insulin resistance can no longer be overcome transition to type 2 diabetes occurs.

2.5. Current Oral Hypoglycaemic Agents

The current therapeutic strategies to treat diabetes are aimed at maintaining glycemic control (FPG between 80-120 mg/dl) and HbA1c levels at or below 7%. Maintenance of HbA1c levels at or below 7% has been shown to decrease the risk of developing microvascular complications (UK Prospective Diabetes Study Group., 1995 & 1998). When proper diet and exercise fail to attain glycemic control the use of anti-diabetic agents becomes necessary. A variety of oral hypoglycemic agents are currently available and these can be generally classified as (1) insulin secretagogues (2) biguanides (3) insulin sensitizers (4) alpha-glucosidase inhibitors or (5) dipeptidyl peptidase-IV (DPP-IV) inhibitors.

The insulin secretagogues include the sulfonylureas and meglitinides and both stimulate insulin release from the pancreas by a common mechanism. Sulfonylureas and meglitinides stimulate insulin secretion by binding to the sulfonylurea receptor of ATP-sensitive K+ channel on β-cells. Meglitinides bind to the sulfonylurea receptor, but also bind to an additional site on the β-cell to induce insulin secretion. Because they secrete insulin independent of glucose concentration, hypoglycemia is a serious side effect of sulfonylureas and meglitinides. Another side effect is their tendency to cause weight gain. This is undesirable especially considering that 80% of diabetics are already overweight. Despite these problems, sulfonylureas are considered a frontline treatment regimen. Meglitinides have similar side effects but they are less pronounced. Some patients do not respond to sulfonylureas while others who have responded may fail to do so after several
years. After 10 years of monotherapy with a sulfonylurea, they generally become ineffective and most patients require a second agent to maintain glucose control (Turner et al., 1999). Some examples of sulfonylureas are given in Figure 1, along with the meglitinide, repaglinide.

![Structure of sulfonylureas and a meglitinide.](image)

<table>
<thead>
<tr>
<th>Drug</th>
<th>R&lt;sup&gt;1&lt;/sup&gt;</th>
<th>R&lt;sup&gt;2&lt;/sup&gt;</th>
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<tr>
<td>Tolbutamide</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;3&lt;/sub&gt;</td>
</tr>
<tr>
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<td>CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>N</td>
</tr>
<tr>
<td>Chlorpropanamide</td>
<td>Cl</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
<tr>
<td>Glybenclamide</td>
<td>Cl</td>
<td>N</td>
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<tr>
<td>Glipizide</td>
<td>H&lt;sub&gt;3&lt;/sub&gt;C&lt;sup&gt;+&lt;/sup&gt;-</td>
<td>N</td>
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Figure 1- Structure of sulfonylureas and a meglitinide.

Biguanides include metformin and phenformin (Figure 2). Their mechanism of action is not completely clear, but it is generally believed that they inhibit hepatic glucose production by decreasing gluconeogenesis, stimulating glycolysis and resensitizing the liver to insulin. They may also resensitize muscle tissue to insulin and decrease intestinal absorption of glucose.

![Structure of Biguanides](image)

<table>
<thead>
<tr>
<th>Drug</th>
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<tr>
<td>Metformin</td>
<td>N–(CH&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
<tr>
<td>Phenformin</td>
<td>NH–(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;–</td>
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Recently, metformin has been shown to increase levels of GLP-1 (Mannucci et al., 2001), a potent endogenous insulinotropic hormone, in obese non-diabetic patients, but its mechanism of action remains controversial. Metformin is presently a frontline treatment option that may be used alone or in combinations with other agents. A beneficial side effect is that it is associated with weight loss, and this makes it preferable to sulfonylureas to treat severely obese diabetics.

Insulin sensitizers include ligands for the peroxisome-proliferator activated receptor γ (PPAR-γ) such as thiazolidinediones (TZD). These drugs enhance insulin sensitivity in adipose, muscle and liver by stimulating the nuclear PPAR-γ receptor, which (1) upregulates proteins required for metabolism of glucose and lipids and (2) activates the glucose transporter gene (GLUT-4) in muscle and adipose tissue. Thiazolidinediones reduce hyperglycemia by increasing cellular glucose consumption, glucose uptake and sensitivity in muscle and adipose tissue. They do not affect insulin levels. PPAR-γ agonists also promote adipocyte differentiation and as a result can cause weight gain as a side effect. To counteract this, dual PPAR-α/γ agonists are being sought. PPAR-α agonists such as fibrates lower lipid triglycerides and raise high density lipoprotein cholesterol (HDLc). They are used to treat hyperlipidemia and are capable of inducing weight loss. The structure of two thiazolidinediones, pioglitazone and rosiglitazone, are shown in Figure 3.
**Figure 3- Structure of thiazolidinediones, rosiglitazone and pioglitazone**

α-Glucosidase inhibitors competitively inhibit the enzymes responsible for conversion of disaccharides to monosaccharides. They prevent the digestion and absorption of carbohydrates because only monosaccharides can be readily absorbed through the gut. α-Glucosidase inhibitors currently available include acarbose, voglibose and miglitol (Figure 4). These drugs are capable of limiting postprandial glucose levels without the risk of hypoglycemia and should be taken with food for optimal effect. They have only modest antidiabetic activity by themselves and are usually used in combination therapy. Side effects include GI disturbances such as flatulence, diarrhea and abdominal pain.

**Figure 4- Structures of α-glucosidase inhibitors**

Combination therapy is an option when one drug is no longer particularly effective. After 3 years monotherapy with either a sulfonylurea or metformin, approximately 50% of patients have HbA1c above 7% and after 9 years this number increases to approximately 75% (Turner et al., 1999). In this case, a second agent of a different class is usually added to the regimen to restore glycemic control through an additive or synergistic effect. The most common combination is metformin with a sulfonylurea. Other useful combinations include metformin and a TZD, metformin with a meglitinide, or an α-glucosidase inhibitor with either metformin or a sulfonylurea. In the case when two agents are no longer effective a third agent of another class might also be added (i.e. TZD to a combination of metformin and a sulfonylurea). Finally, when oral hypoglycemic therapy has failed to achieve therapeutic goals in type 2 diabetes, subcutaneous insulin injections are required to prevent hyperglycemia. These hypoglycemic agents are useful in limiting hyperglycemia, but they do not address
the associated dyslipidemia and atherosclerotic cardiovascular disease, nor do they alter the natural progression of the disease. Therapies which can increase or even preserve β-cell mass would represent a major advance. While a cure is not currently available, research has led to a greater understanding of the etiology of the disease and has resulted in the emergence of novel targets that are being exploited for possible use. GLP-1 based therapy represents such a target and has already been successful.

2.6. Incretin and incretin effect

Eating provokes the secretion of multiple gastrointestinal hormones involved in the regulation of gut motility, secretion of gastric acid and pancreatic enzymes, gall bladder contraction, and nutrient absorption. Gut hormones also facilitate the disposal of absorbed glucose through the stimulation of insulin secretion from the endocrine pancreas. The observation that enteral nutrition provided a more potent insulinotropic stimulus compared with isoglycaemic intravenous challenge led to the development of the incretin concept (Elrick et al., 1964). The first incretin to be identified, glucose-dependent insulinotropic polypeptide (GIP), was purified from porcine intestinal extracts and had weak effects on gastric acid secretion. But more potent insulinotropic actions in human beings (Dupré et al., 1973). GIP is a 42-amino acid hormone synthesised in duodenal and jejunal enteroendocrine K cells in the proximal small bowel.

A second incretin hormone, glucagon-like peptide-1 (GLP-1) was identified after the cloning of the cDNAs and genes encoding proglucagon (figure 5). GLP-1 exists in two circulating equipotent molecular forms, GLP-1(7-37) and GLP-1(7-36) amide, although GLP-1(7-36) amide is more abundant in the circulation after eating. Most GLP-1 is made in enteroendocrine L cells in the distal ileum and colon, but plasma levels of GLP-1, like GIP, also increase within minutes of eating. Hence a combination of endocrine and neural signals probably promote the rapid stimulation of GLP-1 secretion well before digested food transits through the gut to directly engage the L cell in the small bowel and colon.

Plasma levels of GLP-1 are low in the fasted state, in the range of 5–10 pmol/L and increase rapidly after eating, reaching 15–50 pmol/L. The circulating levels of intact GLP-1 and GIP decrease rapidly because of enzymatic inactivation, mainly dipeptidyl peptidase-4 (DPP-4), and renal clearance (Ørskov et al., 1993). Whether additional proteases, such as human neutral endopeptidase, are also essential determinants of GLP-1
inactivation is being investigated. Both GIP and GLP-1 contain alanine at position 2, and hence are excellent substrates for DPP-4. Indeed, DPP-4 is essential for incretin inactivation, and mice with targeted inactivation of the DPP-4 gene have raised levels of plasma GIP and GLP-1, increased insulin secretion, and reduced glucose excursion after glycaemic challenge (Marguet et al., 2000). As a result of DPP-4 activity, intact, biologically active GLP-1 represents only 10–20% of total plasma GLP-1 (Deacon et al., 1995).

**Figure 5- Physiology of GLP-1 secretion and action on GLP-1 receptors in different organs and tissues**

Both GIP and GLP-1 exert their actions by the engagement of structurally distinct G-protein-coupled receptors (GPCRs). The GIP receptor is predominantly expressed on islet β cells, and to a lesser extent, in adipose tissue and in the central nervous system. By contrast, the GLP-1 receptor (GLP-1R) is expressed in islet α and β cells and in peripheral tissues, including the central and peripheral nervous systems, heart, kidney, lung, gastrointestinal tract (figure 5). Activation of both incretin receptors on β cells leads to rapid increases in levels of cAMP and intracellular calcium, followed by insulin exocytosis, in a glucose-dependent manner (Drucker et al., 1987). More sustained incretin receptor signalling is associated with activation of protein kinase A, induction of gene
transcription, enhanced levels of insulin biosynthesis, and stimulation of β-cell proliferation (Drucker., 2006). Both GLP-1R and GIP receptor activation also promote resistance to apoptosis and enhanced β-cell survival, in both rodent (Li et al., 2003) and human islets (Farilla et al., 2003). Consistent with the distribution of GLP-1R expression, GLP-1 also inhibits glucagons secretion, gastric emptying, and food ingestion, and promotes enhanced glucose disposal through neural mechanisms (Burcelin et al., 2001), actions that also contribute to the control of glucoregulation. Notably, effects on glucagon secretion like those on insulin secretory responses, are glucose-dependent, whereas counter-regulatory release of glucagons in response to hypoglycaemia is fully preserved even in the presence of pharmacological concentrations of GLP-1 (Nauck et al., 2002).

Figure 6- Structure of GLP-1, GLP-1R agonists exenatide and liraglutide, and DPP-4 inhibitors vildagliptin and sitagliptin

2.7. GLP-1 signalling

The GLP-1 receptor is coupled to the G-protein Gs, which stimulates the adenylyl cyclase system, thereby increasing intracellular cAMP levels and resulting in the activation of protein kinase A (PKA) and exchange protein directly activated by cAMP (EPAC)
In β-cells, GLP-1 induces $K_{ATP}$ channel closure (through phosphorylation by PKA), followed by membrane depolarisation and an increase in intracellular $Ca^{2+}$, resulting from the opening of $Ca^{2+}$ channels (PKA pathway) and the mobilization of intracellular $Ca^{2+}$ stores (EPAC pathway), ultimately resulting in insulin exocytosis (Ahren., 2006). Furthermore, GLP-1 not only regulates the expression of the insulin gene but also other β-cell genes implicated in insulin secretion, such as those encoding glucokinase and GLUT2. This effect is mediated by increased expression and activity of pancreatic duodenal homeobox-1 (PDX1), which has been shown to be dependent on cAMP/PKA (Wang et al., 2001).

**Figure 7- Signal transduction pathways coupling GLP-1 receptor activation to insulin secretion and β-cell mass protection.** Binding of GLP-1 to its receptor in β-cells results in an increase in intracellular cAMP, leading to the stimulation of insulin exocytosis by two different pathways: PKA-dependent and PKA-independent (EPAC). GLP-1 also increases the activity of PDX1, leading to the regulation of gene expression. The anti-apoptotic effect of GLP-1 is mediated by the activation of CREB via the phosphorylation by PKA and its interaction with a coactivator named TORC2. To migrate into the
nucleus, TORC2 is dephosphorylated via the synergistic activation of TORC2 phosphatase and inhibition of TORC2 kinase, mediated by calcium and cAMP, respectively. Activated CREB enhances gene expression (e.g. IRS-2, which is involved in the activation of PKB). GLP-1 also activates the PI3K pathway by two distinct mechanisms: GLP-1 receptor activation of the Gs protein whereby the subunit dimer Gbg interacts with PI3K; and activation of c-Src followed by the liberation of an endogenous epidermal growth factor (EGF)-like ligand, β-cellulin, which in turn activates the EGF receptor and increases PI3K activity. PI3K subsequently activates its downstream targets MAPK, ERK, PKCz and PKB/Akt. In β-cells, PKC and MAPK activation are associated with GLP-1-induced proliferation, whereas ERK and MAPK activation leads to β-cell differentiation. Stimulation of PKB protects β-cells against apoptosis. AC, adenylyl cyclase; CREB, cAMP response element binding protein; EGF-R: epidermal growth factor receptor; GK: glucokinase enzyme; GLUT2, glucose transporter-2; Ins-insulin; IRS-2, insulin-receptor substrate-2; TORC2, transducer of regulated CREB activity.

The anti-apoptotic effects of GLP-1 are the result of the activation of cAMP and phosphatidylinositol 3-kinase (PI3K) (Figure 7). These two pathways are complementary because inhibition of both pathways produces a greater degree of cell death than does inhibition of either pathway alone (Buteau et al., 2006). Similar cAMP-dependent pathways exist in both insulin-secreting cell lines (Perry & Greig., 2005) and cultured hippocampal neurons (During et al., 2003), favouring cell survival over apoptosis (Perry & Greig., 2005). The cAMP pathway is mediated by the activation of response element binding protein (CREB) and its interaction with the coactivator TORC2 (transducer of regulated CREB activity), thereby enhancing insulin receptor substrate-2 gene expression and leading to protein kinase B (PKB) activation (Jhala et al., 2003) (Figure 7). Moreover, recent data suggest that PKB may modulate proapoptotic (reduction of caspases) and anti-apoptotic (induction of nuclear factor-kB, Bcl2, inhibitor of apoptosis-2) proteins (Hui et al., 2003).

GLP-1 receptor activation leads to the stimulation of PI3K by direct activation of the Gbg dimer or indirect activation of the epidermal growth factor receptor (Buteau et al., 2003) (Figure 7). PI3K subsequently activates its downstream targets: mitogen-activated protein kinases (MAPKs), extracellular-regulated kinases (ERKs), protein kinase C zeta (PKCz)
and PKB/Akt. PKC and MAPK pathways are associated with the GLP-1-induced proliferative signal in β-cells and the trophic effect in cultured neuronal cells. Furthermore, in the pancreas, activation of ERK and MAPK leads to β-cell differentiation, whereas stimulation of PKB protects β-cells against apoptosis induced by elevated glucose and/or fatty acids (Buteau et al., 2004). By inhibiting forkhead transcription factor (FoxO1) through PI3K activation, GLP-1 increases Pdx1 and Foxa2 [proteins are the family of transcription factor that plays important role in regulating genes involved in cell growth, proliferation, differentiation and longevity] expression (Buteau et al., 2006), leading to proliferation (Brubaker et al., 2004) and antiapoptotic effects in the pancreas (Buteau et al., 2006).

2.8. Dipeptidyl-peptidase IV (DPP-4) and DPP-4 inhibitors

Dipeptidyl-peptidase IV (DPP-4) is a ubiquitous enzyme that can be detected in the endothelium of different organs and that is measurable as circulating enzymatic activity in plasma. The incretins, namely glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), are the only substrates of DPP-4 that have been well validated in humans. DPP-4 has also been implicated in the regulation of several additional peptides, such as pituitary adenylate cyclase-activating polypeptide (PACAP) and gastrin-releasing peptide (GRP); however, in humans, these peptides have not been definitively shown to be relevant in vivo substrates for this enzyme (Mest & Mentlein., 2005). DPP-4 cleaves and inactivates GLP-1 within a few minutes (Mentlein., 1999). The mechanism underlying the rapid degradation and elimination of the incretin hormones GLP-1 and GIP has been described elsewhere. DPP-4 preferentially cleaves peptides with the amino acid alanine or proline in position 2 of the N-terminus of the peptide chain. Active GLP-1(7–36) amide is cleaved by DPP-4 to yield a dipeptide (His-Ala) and GLP-1(9–36) amide (Mentlein., 1999, Gault et al., 2002, Knudsen & Pridal., 1996). DPP-4 is also expressed on the cell membrane of activated T lymphocytes as CD26 (De Meester et al., 1999); however, there is no compelling evidence that the catalytic activity of the enzyme is important in immune function. Indeed, in clinical studies with DPP-4 inhibitors, no serious side effects or adverse events on immunological regulatory mechanisms have been observed (Drucker & Nauck., 2006). Recently, 2-year safety data
on sitagliptin from pooled clinical trials have been published (Williams-Herman et al., 2008).

Due to rapid cleavage and inactivation, a therapy with native GLP-1 administered parenterally is not feasible for the continuous treatment of type 2 diabetes, and thus incretin mimetics that are resistant to cleavage by DPP-4 are being pursued. DPP-4 inhibition is an alternate therapeutic option, in that inhibition of this enzyme results in an increase in the circulating levels, of biologically active GLP-1. DPP-4 inhibitors are orally active in contrast to incretin mimetics ((Drucker & Nauck., 2006). Furthermore, they inhibit the degradation of GIP, and potentially other peptides involved in regulating glucose homeostasis. They could therefore have additional beneficial effects in the treatment of diabetes. DPP-4 is a member of a family of endopeptidases, and there is evidence to suggest that selective inhibition of DPP-4 may be important to an optimal safety profile for this new class of anti-hyperglycaemic agents (Lankas et al., 2005). The DPP-4 inhibitors, namely sitagliptin and vildagliptin, are two compounds of the DPP-4 inhibitor class that have been approved in various countries. Alogliptin and saxagliptin are currently under review for approval, and several other DPP-4 inhibitors are in development (Gallwitz., 2008, Deacon., 2008, Huttner et al., 2008). The structures of the DPP-4 inhibitors that have been approved and currently under review are shown in Fig. 8.

2.9. Pharmacology of DPP-4 inhibitors

Sitagliptin, vildagliptin, saxagliptin and alogliptin are competitive inhibitors with high affinity for DPP-4. In humans, the pharmacokinetic and pharmacodynamic properties, efficacy, safety and tolerability have been assessed in numerous clinical studies; the most abundant database is available for sitagliptin and vildagliptin (Ahren., 2008). After a standard meal, active endogenous GLP-1 concentrations are increased two- to threefold by these compounds. Both sitagliptin and vildagliptin have been shown to have clinically meaningful efficacy for the treatment of type 2 diabetes in both monotherapy and in combination with established oral anti-glycaemic agents, including metformin, in studies of at least 6 months duration and up to 2 years for sitagliptin (Ahren., 2008).
Clinical studies to date indicate that DPP-4 inhibition is well tolerated, with an incidence of hypoglycaemia similar to placebo and a neutral effect on body weight (Ahren., 2008). The longest-term clinical experience has been with sitagliptin, and the results of an analysis of pooled safety data from clinical studies of up to 2 years have shown that the incidence rates of adverse experiences overall, including serious adverse experiences, were similar in the sitagliptin and non-exposed groups. Sitagliptin and vildagliptin also have a low propensity to be involved in drug–drug interactions as either a perpetrator or a substrate for metabolism, especially with other anti-hyperglycaemic oral agents (Gallwitz., 2007, Ristic & Bates., 2006).

### 2.10. Development of DPP-4 inhibitors

DPP-4 inhibitors were first pursued as therapeutic agents for the treatment of type 2 diabetes based on the compelling validation of GLP-1 for diabetes therapy as well as on the finding that DPP-4 is the major enzyme involved in the regulation of this incretin hormone (Mentlein et al., 1993, Kieffer et al., 1995, Vilsboll et al., 2003). These observations led to the hypothesis that DPP-4 inhibition would result in higher levels of circulating active GLP-1 (Holst & Deacon., 1998), which was subsequently confirmed when DPP-4 inhibitors were tested in animal models of diabetes, where increased levels of GLP-1, enhanced insulin secretion and improved glucose tolerance were observed (Balkan et al., 1999, Reimer et al., 2002). These findings prompted several pharmaceutical companies to initiate programmes aimed at the identification of DPP-4 inhibitors.
Interest in DPP-4 inhibition, as a new approach to the treatment of type 2 diabetes, was driven not only by the proven glucose-lowering efficacy of GLP-1, but also by the prospects of improvements in safety and tolerability over well-established oral agents. Specifically, because GLP-1 stimulates insulin release and inhibits glucagon production in a strictly glucose-dependent manner, this mechanism was anticipated to have a low risk of hypoglycaemia. Secondly, no weight gain was anticipated with DPP-4 inhibitors. Interest in this class was also driven by an emerging body of evidence that GLP-1 analogues have beneficial effects on β-cell mass in rodents, suggesting the potential of DPP-4 inhibitors for improvements in glycaemic durability.

Extensive structure–activity relationship studies in many different laboratories have resulted in the identification of a number of potent, competitive DPP-4 inhibitors and clinical candidates. Clinical proof of concept for DPP-4 inhibition in diabetic patients was first reported in 2002 with an early inhibitor from Novartis (DPP-728), where significant reductions in fasting plasma glucose and HbA1c were observed in a 4-week study (Ahren et al., 2002).

Sitagliptin was approved in 2006 by the Food and Drug Administration (FDA) for use in monotherapy, and in combination with metformin or a thiazolidinedione. Subsequently, sitagliptin was approved for use with sulphonylurea and sulphonylurea plus metformin. The European Commission has approved the use of: (1) sitagliptin in combination with metformin, sulphonylureas, thiazolidinediones and with metformin plus sulphonylureas and (2) vildagliptin in combination with metformin, sulphonylureas or thiazolidinediones.

2.11. Mechanisms of DPP-4 inhibitor action

DPP-4 has a well-established physiological role in the regulation of the incretin hormones, GLP-1 and GIP. In animals that are genetically deficient in DPP-4, or with pharmacological treatment with a DPP-4 inhibitor, increased active GLP-1, GIP and improved glucose tolerance were observed (Balkan et al., 1999, Reimer et al., 2002, Marguet et al., 2000, Conarello et al., 2003). Increased insulin and decreased glucagon levels were also observed both in DPP-4-deficient mice and, upon pharmacological treatment with inhibitors, in rodents and humans, consistent with the role of this enzyme in incretin regulation and metabolic control. DPP-4 inhibitors do not improve glucose tolerance in mice deficient in both GLP-1 and GIP receptors, indicating that these
incretins are exclusively responsible for the improved glucose tolerance that is observed in these animals (Hansotia et al., 2004). Taken together, these data unequivocally establish that these incretins are endogenous substrates for DPP-4. This enzyme has been implicated in the regulation of peptides in addition to GLP-1 and GIP, including growth-hormone-releasing hormone (GHRH), glucagon-like peptide 2 (GLP-2), pituitary adenylate cyclase-activating polypeptide (PACAP) and gastrin-releasing peptide (GRP) (Mest & Mentlein., 2005, De Meester et al., 2000). Several neuropeptides and chemokines are also in vitro substrates for this enzyme. Although many of these peptides are cleaved efficiently in vitro, it is difficult to determine if these peptides are regulated in vivo by DPP-4, largely because suitable assays for measurement of the endogenous levels of the putative substrates and products are not available. Further work will be required to obtain a comprehensive understanding of the biology of this enzyme. DPP-4-deficient mice are healthy and fertile, and thus if other proteins and/or peptides are regulated by this enzyme, there are no obvious important consequences related to growth and development, reproductive capacity or health. Moreover, the results of clinical studies indicate that selective DPP-4 inhibitors are well tolerated and do not suggest any functions for this enzyme beyond its role in metabolic control (Ahren., 2008).

2.12. DPP-4 inhibitor selectivity

DPP-4 is a member of a family of proteases that includes dipeptidyl-peptidase 8 (DPP8), dipeptidylpeptidase 9 (DPP9) and fibroblast activation protein (FAP) (Sedo & Malik., 2001). DPP8 and DPP9 are ubiquitously expressed and highly conserved across species (Abbott et al., 2000, Ajami et al., 2004). Unlike DPP-4, which is expressed on the cell surface, DPP8 and DPP9 are cytosolic enzymes. No endogenous substrates have been identified, and thus the specific functions of these enzymes are unknown. There is evidence to suggest that selective inhibition may be important to an optimal safety profile with long-term administration of the DPP-4 inhibitor. Early DPP-4 inhibitors with off-target activity against DPP-8 and DPP-9 were shown to cause profound toxicity in preclinical species (Lankas et al., 2005). The results of subsequent toxicity studies with highly selective DPP-4 inhibitors, and an inhibitor of DPP8 and DPP9, demonstrated that the DPP-8/9 inhibitor produced toxicity in preclinical species, but that no toxicities were observed with the selective DPP-4 inhibitor (Lankas et al., 2005)). These results provided
evidence that inhibition of DPP8/9 or a highly related enzyme, but not selective DPP-4 inhibition, is associated with multi-organ toxicities in preclinical species. The relevance of the finding that a DPP8/9 inhibitor produces toxicity in preclinical species to the development of DPP-4 inhibitors for clinical use is unknown.

2.13. Effects of DPP-4 inhibitors on glucagon secretion

After a meal, active endogenous GLP-1 and GIP concentrations are increased two- to threefold by DPP-4 inhibitors. This action leads not only to an increase in insulin secretion as long as hyperglycaemia is present, but also to a suppression of glucagon secretion. Under hypoglycaemic conditions, counter-regulation of glucagon secretion by the alpha cells is not impaired; on the contrary, treatment with a DPP-4 inhibitor may even improve alpha cell sensitivity to glucose (Ahren & Foley., 2003).

2.14. Beta-cell function and beta-cell mass in animal studies with DPP-4 inhibitors

Based on beneficial effects on β-cell mass that have been observed with GLP-1 analogues in rodents, there is interest in knowing if DPP-4 inhibitors are able to influence the disease progression of type 2 diabetes favourably by slowing or even inhibiting loss of beta-cell mass and function. To further understand this potential, animal studies were designed to study the effect of sitagliptin and vildagliptin on islet function and beta cell mass (Pospisilik et al., 2003, Mu et al., 2006). Diabetic mice treated with DPP-4 inhibitors showed a significant, dose-dependent reduction of glycaemic parameters. In these studies, postprandial and fasting hyperglycaemia, HbA1c and lipid parameters (plasma triglycerides and free fatty acids) were improved. Furthermore, DPP-4 inhibitors increased the number of insulin-positive beta cells in islets and the beta-to-alpha cell ratio in different diabetic animals was normalised. Likewise, the islet insulin content was found to be increased and glucose-stimulated insulin secretion in isolated islets was found to be improved in comparison to sulphonylurea-treated mice. In addition, DPP-4-deficient mice are resistant to streptozotocin-induced beta-cell destruction (Conarello et al., 2003). These results have led to the hypothesis that DPP-4 inhibitors may have the potential to delay or prevent disease progression in type 2 diabetes and to improve beta-cell mass and function (Gallwitz., 2007, Ristic & Bates., 2006).

2.15. Drug Profile

2.15.1. Vildagliptin
After 4 weeks of treatment with 100 mg/day, vildagliptin inhibits selectively DPP-4 more than 90%. It is excreted by both digestive and urinary route. Its half-life is shorter than sitagliptin (1.7 vs 12 h). Its action is yet extended in spite of this fast elimination. In monotherapy, it decreases HbA1c in a range of 0.4 to 1.2% vs placebo (De Jager et al., 2007, Ristic et al., 2005). In combination therapy, the decrease of HbA1c was of 1.1% when vildagliptin was added to metformin (Bosi et al., 2007), and of 1.0% when added to pioglitazone. In the meta-analysis from Amori et al. (Amori et al., 2007), HbA1c levels in the different vildagliptin studies varied from +0.40% to -1.20%. In the nine studies comparing vildagliptin to placebo, which gathered 1786 patients, the mean change of HbA1c was -0.73%. Vildagliptin has also been added to insulin (Fonseca et al., 2006): in the combination group compared to the group given insulin alone, mean HbA1c level was reduced by 0.5% vs 0.2%, and hypoglycaemias were less frequent (113 events in 33 patients versus 185 events in 45 patients).

Table 1- Comparison of the Currently Available DPP-4 Inhibitors

<table>
<thead>
<tr>
<th>Mechanisms of action</th>
<th>Sitagliptin</th>
<th>Vildagliptin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Increase of GLP1 level</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increase of insulin secretion</td>
<td></td>
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<tr>
<td></td>
<td>Decrease of glucagon secretion</td>
<td></td>
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<tr>
<td></td>
<td>Preservation of β-cell mass (in vitro)</td>
<td></td>
</tr>
<tr>
<td>Development status</td>
<td>Currently available</td>
<td></td>
</tr>
<tr>
<td>Reduction of HbA1c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- monotherapy</td>
<td>0.6-0.8%</td>
<td>0.4-1.2%</td>
</tr>
<tr>
<td>- in combination with metformin</td>
<td>0.6-0.8%</td>
<td>1.1%</td>
</tr>
<tr>
<td>Effect on weight</td>
<td>Neutral</td>
<td></td>
</tr>
<tr>
<td>Side effects</td>
<td>Upper respiratory tract infections</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Headaches</td>
<td></td>
</tr>
<tr>
<td>Hypoglycaemias</td>
<td>Comparable to placebo</td>
<td></td>
</tr>
<tr>
<td>Lipids</td>
<td>No effect on fasting levels</td>
<td>No effect on fasting levels</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduction of postprandial triglyceride levels</td>
</tr>
<tr>
<td>Precautions for use</td>
<td>Caution in patients &gt; 75 years</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Use not recommended if creatinine clearance &lt; 50 ml/min</td>
<td></td>
</tr>
<tr>
<td>Drug interactions</td>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>

2.15.2. PKF-275-055

The vildagliptin analogue PKF-275-055 was synthesized as a selective, long-acting inhibitor of dipeptidyl peptidase 4 for the treatment of diabetes. The ability of the compound to prevent and improve established peripheral diabetic neuropathy was evaluated in rats with streptozotocin induced diabetes. According to oral glucose tolerance test data, it caused body and muscle weight gain and improved glucose
tolerance when used as a preventative treatment or in established peripheral diabetic neuropathy. While nerve conduction velocity was reduced by 36% at 10 weeks in streptozotocin-treated rats compared with non-diabetic animals, it was only reduced by 17% in animals treated with PKF-275-055. The agent also reduced the decrease in Na+/K+-ATPase activity in diabetic nerves by 50%, partially protected diabetic rats against mechanical hypoalgesia and progressively prevented the increase in hind paw thermal response latencies seen in diabetic animals (Bianchi et al., 2009).