Review Of Literature
2. Review of literature

2.1. Diabetes mellitus

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia and other metabolic derangements. The World Health Organization recognizes three main forms of diabetes: type 1, type 2, and gestational diabetes, which have similar signs, symptoms, and consequences, but different causes and population distributions (Alberti and Zimmet., 1998). Basically, all forms are due to the β-cells of the pancreas being unable to produce sufficient insulin to prevent hyperglycemia. Type 1 is usually due to autoimmune destruction of the pancreatic β-cells. Type 2 is characterized by tissue-wide insulin resistance and varies widely; it sometimes progresses to loss of β-cell function and/or mass. Gestational diabetes is similar to type 2 diabetes, in that it involves insulin resistance in women genetically predisposed to developing this condition. Type 1 and type 2 diabetes are both incurable chronic conditions, but have been treatable since insulin became medically available in 1921 (Dittrich and Dorsche., 1978), and today usually are managed with a combination of dietary treatment, tablets (in type 2) and, frequently insulin replacement therapy. Gestational diabetes typically resolves with delivery. Type 2 diabetes causes many complications. Acute complications (hypoglycemia, ketoacidosis or nonketotic hyperosmolar coma) may occur if the disease is not adequately controlled. Serious long-term angiopathic complications include cardiovascular disease (doubled risk), chronic renal failure (diabetic nephropathy remains the main cause of dialysis in developed world adults), retinal damage (which can lead to blindness and is the most significant cause of adult blindness in non-elderly in the developed world), nerve damage (of several kinds), and other vascular damage that may cause poor healing of wounds, particularly of the feet, can lead to gangrene which can require amputation - the leading cause of non-traumatic amputation in adults in developed world. The major risk factors contributing to the excess of those complications caused by type 2 diabetes include: hyperglycemia, insulin resistance, dyslipidemia, hypertension, smoking, albuminuria, and the procoagulant state (Rother., 2007). Due to the differences in pathogenesis, the treatment strategies also need to be varied. In terms of quantity, the most important complications of type 2 diabetes are macroangiopathies, i.e. myocardial infarction and stroke, which cause some 70 % of
the deaths related to type 2 diabetes. In contrast to microangiopathies (e.g. nephropathy and retinopathy), where the causal relation to hyperglycemia is well supported, the link between hyperglycemia and macroangiopathy is uncertain, at least in terms of the possibility of reducing macrovascular morbidity solely by reducing hyperglycemia. Patients with type 2 diabetes are thus often treated with a lipid lowering statin and an ACE inhibitor or angiotensin receptor antagonist against hypertension and albuminuria in order to achieve adequate reduction of macrovascular risk.

2.2. **Pancreatic islets**

The islets of Langerhans constitute approximately 1-2 % of the mass of the pancreas and they are scattered throughout the entire exocrine parenchyma (Bonner-Weir., 1993). There are about one million islets in a healthy adult human pancreas, with a total weight of ~ 1-1.5 grams (Kulkarni., 2004; Carlsson., 1998), whereas in rats there are 3000-4000 islets in the pancreas (Lifson et al., 1985). Eventhough the number of pancreatic islets is species dependent; their size is fairly constant, ranging between 25-300 μm in diameter. In rodents, each islet contains approximately 2000-4000 cells. Hormones produced in the islets of Langerhans are secreted directly into the blood flow by (at least) four different types of cells: β-cells producing insulin and amylin (65-80 % of the islet cells); α-cells releasing glucagon (15-20 %); δ-cells producing somatostatin (3-10 %); PP-cells containing pancreatic polypeptide (1 %); ε-cells containing ghrelin (Kulkarni., 2004). Islets can influence each other through paracrine and autocrine communication, and β-cells are coupled electrically to adjacent β-cells (but not to other cell types).

The function of the islet endocrine cells is to produce hormones that regulate especially carbohydrate metabolism. The β-cells mainly produce insulin, which lowers glycemia by suppression of hepatic gluconeogenesis and promotion of glucose uptake and storage in for instance skeletal muscle (Zierler., 1999). The α-cells produce glucagon, which increases glucose concentrations by promoting glycogen breakdown in the liver, and thereby protect the body against hypoglycemia. The δ-cells produce somatostatin, which is an inhibitor of the release of other hormones, including insulin and glucagon. The product of PP-cells is mainly released through vagal mediation, and seems to inhibit the exocrine secretions of the pancreas (Balaji et al., 2002; Brissova et al., 2005).
The ε-cells in the islets of Langerhans produce the satiety hormone ghrelin, which recently has been found to regulate glucose-induced insulin release (Dezaki et al., 2006). The mammalian islet has a nonrandom distribution of endocrine cells usually with only β-cells in the center surrounded by a discontinuous mantle of non β-cells (α-, δ-, and PP-cells) (Erlandsen et al., 1976; Orci and unger., 1975) (figure 25).

Figure 25- Distribution of endocrine cells in the pancreas

Human islets have a more complex pattern, can be considered composites of several mantle-core subunits or being lobulated with mantle-core lobules (Erlandsen et al., 1976; Orci, 1976; Grube et al., 1983). Interestingly, in horse this pattern has been reported to be inverted with a central core of α-cells (Fujita, 1973).

2.3. Microcirculation of the islets

The pancreas is part of the splanchnic circulation. The head of the pancreas, which has pancreatic polypeptide-rich and glucagon-poor islets, is supplied by the superior mesenteric artery, while the body and tail, which have glucagon-rich and pancreatic polypeptide-poor islets, are supplied by the celiac artery via the splenic artery (Ohtani et al., 1986; Ohtani, 1983). The interlobular arteries and vein run in parallel and in close proximity to the major ducts. Thereafter, the intralobular arteries pass straight through the center of the lobuli with branches to islets, acini, and ductuli. In some species the intralobular vein is more peripheral and not parallel to the artery (Ohtani et al., 1986;
Ohtani., 1983), whereas in rats the intralobular vessels run parallel to each other (Bonner Weir and Orci., 1982).

The anatomic microvasculature of the pancreatic islets has been extensively studied by dye injection, vascular corrosion casts, in vivo microscopy and non-radioactive microsphere techniques (Bonner-Weir., 1993). In these studies, the islets were found to be substantially more vascularized than the exocrine tissue and to be filled first with infusion, and actually have a direct arteriolar blood flow as well (Figure 26).

Rodents have a well-developed insulo-acinar portal system that is similar to humans (Murakami et al., 1992; Wharton., 1932). In rodents, the flow pattern is from centre towards the periphery (Menger et al., 1992). The microvasculature pattern may vary depending on the size of the islet in the rat (Bonner-Weir., 1982). Small islets (< 160 μm in diameter) usually have one arteriole and empty into several small efferent venules that are either connected to exocrine capillary plexa, thereby forming an insulo-acinar portal system, or empty into large veins in the exocrine parenchyma. Islets exceeding 160 μm in diameter receive an arterial blood supply from one to three arterioles and the efferent
Collecting venules drain into large veins without forming any insulo-acinar system (Bonner-Weir., 1982) (Figure 27).

Figure 27- Vascular organization in a large islet. (Adopted from Bonner-Weir and Orci, Diabetes 31:883, 1982)

The islet capillaries have been shown to be 10 times more fenestrated than the capillaries in exocrine parenchyma (Henderson and Moss., 1985). These fenestrations and high vascular density are dependent on vascular endothelial growth factor (VEGF), which is produced by the islet cells (Kamba et al., 2006; Lammert et al., 2003).

Since the cell types have specific locations within the islet, the pattern of blood flow through the islet has been proposed to have a significant impact on the ability of cells to intercommunicate within the islet (Figure 28).
Figure 28- Islet blood flow regulatory sites (Brunicardi et al, Diabetes 45:385, 1996).

This has given rise to three competing models of islet microcirculation. The first model, using scanning electron microscopy of corrosion casts, describes that non β-cells are perfused before β-cells (Murakami and Fujita., 1992; Murakami et al., 1993). The second model is based on morphological and physiological studies using corrosion casts or anterograde and retrograde pancreas perfusions with anti-insulin/anti-somatostatin gammaglobulin (Samols et al., 1988). These studies suggest that β-cells are perfused before non β-cells in both rodents (Samols et al., 1988) and humans (Stagner and Samols., 1992). Model three describes a gated portal pattern from the afferent to efferent pole of the islet based on intravital microscopical studies (Brunicardi et al., 1996; Liu et al., 1993; McCuskey and Chapman., 1969).

Non-radioactive microsphere techniques allow a continuous study of the islet blood flow during prolonged time periods, and have been extensively used to study islet blood flow in experimental animals (Jansson., 1994). In rats, the islets have been shown to receive 5-10 % of the total pancreatic blood flow (Atef et al., 1997; Jansson and Hellerstrom., 1983), 2-5 % in mice (Carlsson et al., 1996) and 15-20 % in rabbits (Lifson et al., 1980), even though they only compose 1-2 % of the total pancreatic volume. The small islets receive only a minor part of the total islet blood flow within the pancreas (Lifson et al., 1985), and then empty out the exocrine capillary system (Bonner-Weir., 1993). This means that the flow to these islets is regulated by mechanisms common to those of the whole pancreatic gland. Because of the small fraction of the whole islet blood flow which is diverted to these islets, they are probably of minor importance for whole pancreatic islet blood flow. The larger islets, which receive the major part of the blood flow (Lifson et al., 1985), are supplied by one or three afferent arterioles each. Transplanted isolated pancreatic islets show high basal blood flow values.
similar to that of the islets within the normal pancreas (Jansson and Sandler., 1990; Jansson and Sandler., 1992; Sandler and Jansson., 1987). This means that islet blood flow can be regulated separately from the flow to the whole pancreas. An appropriate blood perfusion is essential for maintenance of islet metabolism and insulin secretion. Normally, the blood flow to endogenous islets is tightly regulated at the arteriolar level by nervous, endocrine and metabolic mechanisms (Carlsson et al., 1997). Islet blood flow can be preferentially increased from ~ 10% in a dose-dependent and time-dependent manner after glucose administration (Jansson., 1984).

Nonetheless, this increased blood perfusion seems to appear only in those islets that have the highest basal blood perfusion (Carlsson et al., 2002; Jansson., 1996). The vagus nerve is the most important mediator of this increase in islet blood flow, which is influenced by glucose sensing mechanisms in the brain, oral cavity, and duodenum (Carlsson et al., 1999; Jansson and Hellerstrom., 1986). Furthermore, similar glucose sensors are present in the liver; however, their regulation of islet blood flow is mediated by sympathetic nerves (Carlsson et al., 2000). Islet blood flow can be also regulated by local substrates, most notable are adenosine (Carlsson et al., 2002) and nitric oxide (NO) (Svensson et al., 1994).

2.4. 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 insulino tropic 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 adenyl 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., 2007, Deacon., 2008, Huttner et al., 2008). The structures of the DPP-4 inhibitors that have been approved and currently under review are shown in Figure 29.

2.5. 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).

![Figure 29- Structural formulas of sitagliptin, vildagliptin, saxagliptin and alogliptin](image)

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).

2.6. **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. 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).