PART-A

THE MECHANISTIC STUDIES OF TAMARIND SEED EXTRACT ON GLUCOSE HOMEOSTASIS, PANCREATIC BETA CELL DAMAGE, GENES AND PROTEIN EXPRESSION IN NIDDM RATS
INTRODUCTION
1. Introduction

Diabetes mellitus (DM) is a common metabolic disease characterized by elevated blood glucose levels, resulting from inadequate or no pancreatic insulin secretion, with or without concurrent impairment of insulin action. This illness affects approximately 150 millions of people worldwide and its incidence rate is expected to double during the next 20 years (Cohen & Goedert, 2004). The prevalence of diabetes mellitus (DM) is growing rapidly worldwide and is reaching epidemic proportions (King & Rewers, 1991; Bjork, 2003). It is estimated that there are currently 285 million people with diabetes worldwide and this number is set to increase to 438 million by the year 2030. There is also consensus that the South Asia region will include three of the top ten countries in the world (India, Pakistan and Bangladesh) in terms of the estimated absolute numbers of people with diabetes (Sicree, 2009). Although the exact reasons why Asian Indians are more prone to type 2 diabetes at a younger age and premature cardiovascular disease (CVD) remain speculative, there is a growing body of evidence to support the concept of the “Asian Indian Phenotype”. This term refers to the peculiar metabolic features of Asian Indians characterized by a propensity to excess visceral adiposity, dyslipidaemia with low HDL cholesterol, elevated serum triglycerides and increased small, dense LDL cholesterol, and an increased ethnic (possibly genetic) susceptibility to diabetes and premature coronary artery disease (Deepa et al., 2006; Joshi, 2003). Ancient Indian texts make mention of the disease “Madhumeha” which would correspond to the modern term “Diabetes mellitus”, suggesting that diabetes must have been present in India even before 2500 BC. Although, there is no evidence as to how prevalent the condition was, a recent article hypothesizes that it could have been quite common in India, even in ancient times (Weaver & Narayan, 2008).

1.1. 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.

Fig. 1. Insulin release and action (Notkins, 2002).

1.2. Causes of T2DM

T2DM as a common and complex disease has been characterized by the following causes:

- Obesity: obesity is also considered a key risk factor for T2DM. The association between increasing body mass index (BMI) and greater weight gain and risk of diabetes is most pronounced among Asians, suggesting that lower cut off BMI values are needed to identify Asians at a higher risk of diabetes (Shai et al., 2006). BMI cut point for Indians
for any cardiometabolic risk factors is 23 kg/m2 in both sexes, whereas that of waist circumference (WC) is 87 cm for men and 82 cm for women (Mohan et al. 2007).

- Abdominal adiposity: there is also a probable indication that there is a preferential abdominal adiposity in Indians irrespective of the degree of general adiposity (Ramachandran et al., 2002).

- Imbalance of human metabolism is associated with T2DM: Changes in work patterns from heavy labour to sedentary, the increase in computerization and mechanization, and improved transport are just a few of the changes that have had an impact on human metabolism (Zimmet et al., 2001).

- Genes: since 2007, genome-wide association studies has catalogued around 20 genes (like TCF7L2, HHEX, CDKAL1, SLC30A8 etc.) showing a strong association (with modest odds ratio ranges between 1.2 to 1.5) with T2DM (Sladek et al., 2007, WTCCC 2007, Scott et al., 2007).

- Ethnicity: the interethnic differences (like differences in prevalence of T2DM among Europeans, Americans, Chinese, and Asian Indians) in insulin resistance may have an environmental or genetic explanation. The main acquired factors that seemingly increase insulin resistance in all ethnic groups include obesity, sedentary lifestyle, diet rich in animal products, and aging (Abate & Chandalia, 2001).

1.3. Complications of diabetes

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).

1.4. Epidemiology of diabetes

The press release on 14 NOVEMBER - World Diabetes Day 2011 pointed out that, the new figures indicate the number of people living with DM is expected to rise from 366 million in 2011 to 552 million by 2030, if no urgent action is taken. This equates to approximately three new cases every ten seconds or almost ten million per year. In addition, report said, in some of the poorest regions in the world such as Africa, where infectious diseases have traditionally been the focus of health care systems, diabetes cases are expected to increase by 90% by 2030. At least 78% of people in Africa are undiagnosed and do not know they are living with diabetes. In addition, few clinical reports released by International Diabetes Federation are as follows (2011)

- 80% of people with diabetes live in low and middle income countries.
- 78,000 children develop type 1 diabetes every year
- The greatest number of people with diabetes are between 40-59 years of age

DM is recognized by the World Health Organization (WHO) as a growing worldwide epidemic with more than 171 million people worldwide (2.8%) afflicted in 2000 and it is conservatively estimated that the number will more than double to 366 million (4.4%) by 2030 (Wild et al., 2004). The WHO predicts that diabetes mellitus will become one of the
world's leading causes of death and disability within the next quarter century (World Health Organization Fact Sheet N° 236., 2006). In 2005, it was estimated that between 1.1-2.9 million people died from diabetes and its complications, making it the fifth leading cause of death in the world (Roglic et al., 2005).

Type 2 DM accounts for 90-95% of all cases of diabetes and is largely associated with obesity and physical inactivity, which have been shown to lead to insulin resistance. In fact, obesity is the greatest risk factor and it is estimated that 80% of diabetics are overweight (Triplitt et al., 2006). The increase in this global phenomenon has been largely attributed to the spread of the "western lifestyle", which refers to the combined detrimental effects of decreased exercise and unhealthy diet. In terms of the total number of people afflicted, the top three countries are India, China and the United States (US) (Wild et al., 2004). In India, 31.7 million people had diabetes in 2000 but this number is expected to skyrocket to 79.4 million by 2030. In China, 20.8 million had diabetes in 2000 increasing to 42.3 million by 2030. 17.7 million people had diabetes in the US in 2000 and it is predicted to rise to 30.3 million by 2030.

Type 2 DM is part of the "metabolic syndrome", also referred to as syndrome X, which includes a set of disorders characterized by obesity, insulin resistance, hypertension and dyslipidemia. It is a chronic metabolic disorder that even with current therapies progressively worsens with time and some of its complications include retinopathy, nephropathy, neuropathy and atherosclerotic cardiovascular disease (i.e. stroke, heart attack and foot ulcers).

Insulin resistance and β-cell failure underlie the disease. In the initial stages of insulin resistance, glucose homeostasis can be maintained through hyperinsulin secretion by β-cells. Overt diabetes only occurs when β-cells can no longer compensate for insulin resistance. It is reported that newly diagnosed patients with type 2 diabetes mellitus have approximately 50% β-cell function (UK Prospective Diabetes Study Group., 1995), due in part to a 30% reduction in β-cell mass. In this study, an increase in fasting plasma glucose levels in patients treated by diet alone or with sulfonylurea therapy after six years as associated with a decline in insulin levels due to progressive β-cell dysfunction. Therefore, amelioration of the decline in β-cell function is critical in altering the progressive nature of the disease. Unfortunately, there is no therapy aimed at preserving β-cell function.
There is no cure for diabetes, but the progression of the disease may be slowed down considerably through proper diet and regular physical activity. Present treatment is aimed at maintaining strict glycemic control and while some patients may be managed by diet and exercise, more typically, one or a combination of oral hypoglycemic agents are required for effective glycemic control. However, even with current pharmacological treatment the disease progressively worsens with time. For these reasons, the development of new drugs is actively being pursued.

The United Nations (UN) has recognized the diabetes epidemic as a threat to the entire world and in an effort to raise public awareness has declared November 14 (beginning in 2007) as World Diabetes Day (United Nations: General Assembly., 2007). Aside from the human pain and suffering, the financial burden that this disease places on economic development throughout the world is enormous. The total cost in 1997 in the US alone has been estimated at $98 billion (World Health Organization Fact Sheet N° 236., 2006). This includes $44 billion in direct healthcare costs and another $54 billion in indirect loss of productivity. In 2002, the cost increased to $132 billion and is estimated to rise to $192 billion in 2020 (American Diabetes Association., 2003). Health-care costs for nations range from 2.5-15% of annual health care budgets (World Health Organization Fact Sheet N° 236., 2006). Because of the enormous scale of the disease, cost-effective therapies will be required to treat people, especially those from poorer developing parts of the world that cannot afford expensive medications. The solution to this problem is certainly complex and will require a novel and concerted global effort that combines modern "western" medicine with alternative traditional systems used throughout many parts of the world.

1.5. Overview of interrelationship between oxidative stress and Diabetes

Oxidative stress corresponds to an imbalance between the rate of oxidant production and that of their degradation. Aerobic organisms such as vertebrates and man in particular produce their energy from the oxidation of organic substrates by molecular oxygen. The complete four-electron reduction of molecular oxygen occurs within mitochondria and produces water, at the end of the respiratory chain. Sometimes molecular oxygen is partly reduced instead of the proteins of the respiratory chain, and superoxide and various reactive oxidant intermediates are produced, leading to secondary oxidations and tissue insults. Excessively high levels of these free radicals cause damage to cellular proteins, membrane lipids and nucleic acids, and eventually cell death. Various mechanisms have been suggested to contribute to the formation of these reactive oxygen-free radicals. Glucose oxidation is
believed to be the main source of free radicals. In its enediol form, glucose is oxidized in a transition-metal dependent reaction to an enediol radical anion that is converted into reactive ketoaldehydes and to superoxide anion radicals. The superoxide anion radicals undergo dismutation to hydrogen peroxide, which if not degraded by catalase or glutathione peroxidase, and in the presence of transition metals, can lead to production of extremely reactive hydroxyl radicals (Jiang, 1990; Wolff & Dean, 1987). Superoxide anion radicals can also react with nitric oxide to form reactive peroxynitrite radicals (Halliwell & Gutteridge, 1990; Hogg, 1993). Hyperglycemia is also found to promote lipid peroxidation of low density lipoprotein (LDL) by a superoxide-dependent pathway resulting in the generation of free radicals (Tsai et al., 1994; Kawamura et al., 1994). Another important source of free radicals in diabetes is the interaction of glucose with proteins leading to the formation of an Amadori product and then advanced glycation endproducts (AGEs) (Hori et al., 1996; Mullarkey et al., 1990). These AGEs, via their receptors (RAGEs), inactivate enzymes and alter their structures and functions (McCarthy et al., 2001), promote free radical formation (Baynes, 1991 & 1999), and quench and block antiproliferative effects of nitric oxide (Vlassara, 1997; Wautier et al., 1994). By increasing intracellular oxidative stress, AGEs activate the transcription factor NF-kB, thus promoting up-regulation of various NFkB controlled target genes (Mohamed et al., 1999). NF-kB enhances production of nitric oxide, which is believed to be a mediator of islet β-cell damage.

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).

Fig. 2. Overview of free radical and ROS formation and elimination. The main pathway of free radical and ROS formation starts with the partial reduction of molecular oxygen to superoxide by oxidases or the reduced or semiquonone forms of ubiquonole (coenzyme Q), the hydrogen peroxide produced by dismutation of superoxide can decompose in the presence of transition metals to give rise to hydroxyl radicals, an extremely instable oxidant. The latter can initiate lipid peroxidation in the presence of oxygen. Another strong oxidant peroxynitrite is formed by reaction between superoxide and nitric oxide, an intracellular messenger formed from arginine, NADPH and oxygen.

Abbreviations: 6PGL- 6 phosphogluconolactone; A, oxidised substrate; AH₂, reduced substrate; Arg, arginine; ASC, Ascorbate; ASC*, semidehydroscrbate; Cat, catalase; Cit, Citrulline; DHA, dehydroascorbate; G6-P, glucose 6 phosphate; GSH, reduced glutathione; GSSG, oxidised glutathione; GSR, glutathione reductase; HK, hexokinase; L*, Lipid; LOH, an hydroxylated lipid; LOO*, A lipid peroxy radical; LOOH, A lipid hydroperoxide; MPO, Myeloperoxidase; Ps, Photosensitiser, Q, Ubiquonone; QH*, Ubisemiquonone; Q
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 free fatty acids contribute to β-cell malfunction, in a condition called glucomlipotoxicity. When insulin resistance can no longer be overcome, transition to type 2 DM occurs.

1.6. Biological relevance of markers of oxidative stress and Diabetes

Possible sources of oxidative stress in diabetes include decreased level of antioxidant enzyme activities and increased generation of reactive oxygen species (ROS), which leads to lipid peroxidation and glycation. While, the biochemical significance of oxidative stress has been understood for some time, the estimation of oxidative stress in vivo is quite difficult (Favier, 1997) with no standardization available. The measurement of antioxidant enzymes activities namely superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) and malondialdehyde (MDA), a parameter of lipid peroxidation have been acknowledged as tools for the assessment of oxidative damage in vivo (Westie, 2000; Favier, 1997).

The primary ROS produced in the course of oxygen metabolism is superoxide, which is a highly reactive, cytotoxic ROS. Superoxide is dismutated to a far less reactive product, hydrogen peroxide (H₂O₂), by a family of metalloenzymes known as SOD (Vaziri et al, 2003). The ubiquitous SODs catalyze the disproportionation of superoxide to molecular oxygen and peroxide and thus are critical for protecting the cell against the toxic products of aerobic respiration.

Thus, SOD is the front line of defence against ROS-mediated injury. GSH is by far the most important antioxidant in most mammalian cells. This ubiquitous tripeptide, γ-Glu-CysGly, performs many cellular functions. In particular, the thiol containing moiety is a potent reducing agent. (Apel & Hirt, 2004) Intracellular GSH is converted to GSSG by selenium-containing GSH peroxidase, which catalyzes the reduction of H₂O₂ in the presence of GSH and GSH peroxidase is coupled with oxidation of glucose-6-phosphate and of 6-phosphogluconate, which provides NADPH for reduction of GSSG by GSSG reductase. This is a major pathway of H₂O₂ metabolism in many cells. It is thus important for the protection of membrane lipids against oxidation. Intermediates such as O₂ and H₂O₂ are formed extensively in biological systems, and these produce reactive oxygen species that can lead to
organic peroxide formation. GSH has the important function of destroying reactive oxygen intermediates and free radicals that are constantly formed in metabolism (Meister, & Anderson, 1983).

Catalase (CAT, H$_2$O$_2$:H$_2$O$_2$ oxidoreductase) is an enzyme that decompose hydrogen peroxide (H$_2$O$_2$) to molecular oxygen (O$_2$) and water (H$_2$O). This activity of catalase is known as catalytic activity. It also exhibits peroxidatic activity and catalyses the oxidation of various hydrogen donors in the presence of relatively lower concentrations of hydrogen peroxide (Cetin et al., 1997).

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2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2
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H_2O_2 + GSH \rightarrow H_2O + GSSG
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\[
GSSG + NAD(P)H \rightarrow GSH + NAD(P)^+
\]
\[
CAT + H_2O_2 \rightarrow (CAT - H_2O_2) \text{ complex-1}
\]
\[
(CAT + H_2O_2 + H_2O_2) \rightarrow CAT + 2H_2O + O_2 \text{ (catalytic activity)}
\]
\[
(CAT + H_2O_2 + AH_2) \rightarrow CAT + 2H_2O + A \text{ (peroxidative activity)}
\]

**Fig. 3. Cascade of reaction for breakdown of free radical in living organism**

1.7. **Role of inflammatory cytokine and adiponectin in type-2 diabetes**

Insulin affects cells through binding to its receptor on the surface of insulin-responsive cells. The stimulated insulin receptor phosphorylates itself and several substrates, including members of the insulin receptor substrate (IRS) family, thus initiating downstream signaling events (White, 1997; Saltiel & Pessin, 2002). The inhibition of signaling downstream of the insulin receptor is a primary mechanism through which inflammatory signalling leads to insulin resistance. Exposure of cells to TNF-α or elevated levels of free fatty acids stimulates inhibitory phosphorylation of serine residues of IRS-1 (Yin et al., 1998). This phosphorylation reduces both tyrosine phosphorylation of IRS-1 in response to insulin and the ability of IRS-1 to associate with the insulin receptor and thereby inhibits downstream signalling and insulin action (Hotamisligil et al., 1996). Insulin has a regulatory effect on FFA metabolism. A defect in the ability of insulin to regulate the FFA metabolism could contribute to increase FFA levels (Fig. 3).

The adiponectin secretes a number of peptides named adipocytokines or adipokines. The most abundantly secreted adipokine is adiponectin and its action leads to activation of AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor. These
in intracellular pathways are involved in fatty-acid oxidation and glucose uptake and suggest a role of adiponectin as an endogenous insulin sensitizer (Yamauchi et al., 2003). A huge number of studies have appeared on the correlation between adiponectin, type 2 diabetes, coronary artery disease and diet-induced obesity, with conclusion that down-regulation of adiponectin in all these conditions, has been suggested to contribute to the pathogenesis of these diseases. Furthermore, insulin-sensitizing action of adiponectin is through activation of AMPK in the peripheral tissues, which include stimulation of fatty acid oxidation and glucose uptake in skeletal muscle and suppression of glucose production in the liver (Fang & Sweeney, 2006).

Fig. 4. Mechanism for adipocytokine effects on liver, pancreas, heart, muscle arteries and platelets (Fang & Sweeney, 2006)

1.8. Diabetes and glucose transporters

Glucose derived from the diet is transferred from the lumen of the small intestine, and both dietary glucose and glucose synthesised within the body have to be transported from the circulation into target cells. These processes involve the transfer of glucose across plasma membranes and this occurs via integral transport proteins.
(Antihyperglycemic Activity of Tamarind Seeds)

1. Introduction

(i) The Na\(^+\)-dependent glucose co-transporters (SGLT, members of a larger family of Na-dependent transporters, gene name SLC5A) (Wright, 2001).

(ii) The facilitative Na\(^+\)-independent sugar transporters (GLUT family, gene name SLC2A) (Mueckler, 1994)

The facilitative transporters (GLUT) utilise the diffusion gradient of glucose (and other sugars) across plasma membranes and exhibit different substrate specificities, kinetic properties and tissue expression profiles.

GLUT-1 is expressed particularly in the brain (including the blood–brain barrier) and erythrocytes. Moderate levels of expression are also observed in adipose tissue, muscle and the liver. GLUT-2 is expressed primarily in pancreatic β-cells, the liver and the kidneys. In the β-cells, GLUT-2 is thought to play a role in the glucose-sensing mechanism, while in the liver it is expressed on the sinusoidal membrane of hepatocytes and allows for the bidirectional transport of glucose under hormonal control. GLUT-2 is also found on the basolateral surface of proximal renal tubules and enterocytes, where it forms part of the transcellular pathway for glucose and fructose transport. GLUT-3 has a high affinity for glucose and this is consistent with its presence in tissues where the demand for glucose as a fuel is considerable, in particular the brain. The insulin-responsive glucose transporter, GLUT-4, is found in heart, skeletal muscle and adipose tissue, where it is responsible for the reduction in the postprandial rise in plasma glucose levels; it is also found in the brain (Rayner et al., 1994).

Insulin-resistant glucose utilization in peripheral tissues such as muscle and adipose tissues is a universal feature of both insulin-dependent (IDDM) and non-insulin-dependent (NIDDM) diabetes mellitus. GLUT expression is down regulated when there is relative insulin deficiency, such as in STZ induced diabetes (Charron, 1999). In this process, GLUT, SREBP-1c along with other components plays crucial role. (Charron et al., 1989). SREBP-1c regulates the transcription of genes involved in cholesterol and fatty acid metabolism (Ayala et al., 2009). SREBP-1c expression and nuclear abundance were low in the liver of STZ-induced diabetic rats, and markedly increased after insulin treatment (Shimomura et al., 1999).

1.9. 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 (Fig. 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 (Orskov 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).

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 (Fig. 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).

Fig. 5. Physiology of GLP-1 secretion and action on GLP-1 receptors in different organs and tissues (Drucker, 2006)
1.10. Current Oral Hypoglycaemic Agents

The current therapeutic strategies to treat diabetes are aimed at maintaining glycemic control (FPG between 80-120 mg/dl) and glycated haemoglobin (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) α-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 Fig. 6, along with the meglitinide, repaglinide.

Biguanides include metformin and phenformin (Fig. 7). 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 resenzitizing the liver to insulin. They may also resensitize muscle tissue to insulin and decrease intestinal absorption of glucose.

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 $\gamma$ (PPAR-$\gamma$) such as thiazolidinediones (TZD). These drugs enhance insulin sensitivity in adipose, muscle and liver by stimulating the nuclear PPAR-$\gamma$ 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-$\gamma$ agonists also promote adipocyte differentiation and as a result can cause weight gain as a side effect. To counteract this, dual PPAR-$\alpha/\gamma$ agonists are being sought. PPAR-$\alpha$ 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 Fig. 8.

One of the therapeutic approaches for reducing postprandial hyperglycemia in patients with DM is to prevent absorption of carbohydrates after food uptake. Only monosaccharides, such as glucose and fructose, can be transported out of the intestinal lumen into the bloodstream. Complex starches, oligosaccharides, and disaccharides must be broken down into individual monosaccharides before being absorbed in the duodenum and upper jejunum. This digestion is facilitated by enteric enzymes, including pancreatic α-amylase, and α-glucosidases that are attached to the brush border of the intestinal cells.

α-glucosidase inhibitors delay absorption of complex carbohydrates and thus inhibit postprandial glucose peaks thereby leading to decreased postprandial insulin levels. Currently, four α-glucosidase inhibitors exist: acarbose, miglitol, voglibose and emiglitate (Fig. 9). Of these, acarbose is by far the most prescribed drug. In most guidelines it is not a drug of first choice but used as an addition to other drugs for type 2 diabetes when treatment goals are not met, or in case of contra-indications for other medications (EDPG, 1999). 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.

![Fig. 8. Structure of thiazolidinediones, rosiglitazone and pioglitazone](image-url)
Fig. 9. Structures of α-glucosidase 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).

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.