SECTION-A

INTRODUCTION
1 INTRODUCTION
1.1 Diabetes Mellitus

Diabetes mellitus is a serious chronic metabolic disorder that has significant impact on the health, quality of life, and life expectancy of the affected, as well as on the health care system. The World Health Organization (WHO), has projected that the global prevalence of type 2 DM will more than double from 135 million in 1995 to 300 million by the year 2025 (Horton 2008). Very disturbing estimates were reported by International Diabetes Federation, that in the year 2002, at least 177 million people were having DM worldwide, which indicates that previous estimate of 225 million by 2010 is an underestimate (Cosgrove, Engelgau et al. 2002). The greatest increase will be in India from 19.4 million to 57.2 million, while in China from 16 million to 37.6 million and USA from 13.9 million to 21.9 million during the same period, unless effective preventive measures are implemented to control this enormous increase. Currently India has got the largest number of diabetics and is being called as diabetic capital of the world. The prevalence of type 2 diabetes particularly in women is increasing worldwide, although it is more pronounced in low-income countries. This increase is mostly due to population, ageing and the ubiquitous rise in prevalence of obesity (Roglic 2009). Diabetes mellitus has been divided into two major categories: type 1 diabetes (formerly known as insulin-dependent diabetes mellitus or IDDM) and type 2 diabetes (formerly known as non-insulin dependent diabetes mellitus or NIDDM).

1.2 Diagnostic Criteria for Diabetes Mellitus

Diagnostic criteria, which clinically establish that an individual is suffering from diabetes mellitus include:

- Having a fasting plasma glucose level in excess of 126 mg/dl (7mM). Normal levels should be less than 100 mg/dl (5.6 mM) or
- Having plasma glucose levels in excess of 200 mg/dl (11mM) at two time points during an oral glucose tolerance test, OGTT, one of which must be within 2 hrs of ingestion of glucose.

The earlier a person is diagnosed with diabetes (principally type 2 diabetes), the better chance the person has of staving off the primary negative consequences which are renal failure, blindness and limb amputations due to circulatory problems. The American Diabetes Association (ADA) is planning to recommend that physicians consider subjects to be pre-diabetic if their fasting blood glucose level is 100-125 mg/dl (5.6-6.9 mmol/L)
and whose glucose level are 140-199 mg/dl (7.8-11.0 mmol/L) following an oral glucose tolerance test (OGTT) and if blood glucose level in diabetic patients is \( \geq 200 \) mg/dl (11.1 mmol/L) following an oral glucose tolerance test then they should be considered to have frank diabetes.

**Table:** Diagnostic criteria for diabetes mellitus and Prediabetes: impaired fasting glucose and impaired glucose tolerance

<table>
<thead>
<tr>
<th>Category</th>
<th>Fasting plasma glucose mg/dl (mmol/L)</th>
<th>2 h plasma glucose mg/dl (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt;100 (5.6)</td>
<td>&lt;140 (7.8)</td>
</tr>
<tr>
<td>Impaired fasting glucose</td>
<td>100-125 (5.6-6.9)</td>
<td></td>
</tr>
<tr>
<td>Impaired glucose tolerance</td>
<td></td>
<td>140-199 (7.8-11.0)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>( \geq 126 ) (7.0)</td>
<td>( \geq 200 ) (11.1)</td>
</tr>
</tbody>
</table>

1.3 Types of Diabetes

Diabetes mellitus is a heterogeneous metabolic disorder that occurs due to numerous reasons. Two main classifications of diabetes mellitus exist, idiopathic and secondary. Idiopathic diabetes is divided into two main types: Type 1 diabetes mellitus and Type 2 diabetes mellitus.

1.3.1 Type 1 Diabetes

1.3.1.1 Pathogenesis

Type 1 diabetes is characterized by autoimmune destruction of insulin-producing \( \beta \) cells in the pancreas by CD4+ and CD8+ T cells and macrophages infiltrating the islets. The disease accounts for about 10% of all cases of diabetes, occurs most commonly in people of European descent and affects 2 million people in Europe and North America. There is a marked geographic variation in incidence, with a child in Finland being about 400 times more likely than a child in Venezuela to acquire the disease. The current global increase in incidence of 3% per year is well reported and it is predicted that the incidence of type 1 diabetes will be 40% higher in 2010 than in 1998 (Onkamo, Vaananen et al. 1999). This rapid rise strongly suggests that the action of the environment on susceptibility genes contributes to the evolving epidemiology of type 1 diabetes. Before safe and rational therapies can be offered in a clinical setting, a detailed understanding of the immune-mediated process that results in type 1 diabetes is required, as is the accurate identification of those at risk of the disease.
1.3.1.2 Prevention of Type 1 Diabetes

The presence of β-cells in patients with long-standing type 1 diabetes, despite ongoing autoimmunity, implies that new formation of β-cells may be occurring (Meier, hushan et al. 2005). Although an ambitious aim currently, targeted regeneration of such β-cells offers another strategy to prevent type 1 diabetes. Regeneration of β-cells is therefore an area of major active investigation, with recent studies reporting differentiation of pancreatic and non-pancreatic progenitors as well as replication of existing islet β-cells. One study has shown that a single murine adult pancreatic precursor exists that can differentiate into cells with the characteristics of islet β-cells (Seaberg, Smukler et al. 2004).

1.3.1.3 Future of Type 1 Diabetes:

There are still some ways from developing a pill to prevent type 1 diabetes, but all the divergent strands of ongoing research, from epidemiology to molecular biology, immunology to clinical trials, appear to be converging to provide clear perspectives on the therapeutic interventions that are most likely to be successful. Two strategies are open to physicians who have patients with type 1 diabetes: the first is to prevent initiation of autoimmunity; the second is to reverse the effects of ongoing autoimmunity coupled with β-cell regeneration. Although highly ambitious, the prevention of type 1 diabetes could be possible by identifying and eliminating environmental risk factors. The next line of defense would be to re-educate the immune system through exposure to β-cell antigens with the use of oral or nasal tolerance strategies (Skyler, Greenbaum et al. 2008). The observation that insulin may be the primary auto-antigen provides support for therapies using insulin to induce tolerance.
1.3.2 Type 2 Diabetes

Type 2 diabetes mellitus constitutes more than 90% of total diabetes cases. This heterogeneous disorder afflicts an estimated 6% of the adult population in Western society. Its worldwide frequency is expected to continue to grow by 6% per annum, potentially reaching a total of 200-300 million cases in 2010 (Kahn and Flier 2000). The main force driving this increasing incidence is a staggering increase in obesity, the single most important contributor to the pathogenesis of diabetes. Type 2 diabetes is known to have a strong genetic component with contributing environmental determinants. Although the disease is genetically heterogeneous, there appears to be a fairly consistent phenotype once the disease is fully manifested. Whatever the pathogenic causes, the early stage of type 2 diabetes is characterized by insulin resistance in insulin-targeted tissues, mainly the liver, skeletal muscle, and adipocytes. Insulin resistance in these tissues is associated with excessive glucose production by the liver and impaired glucose utilization by peripheral tissues, especially muscle. These events undermine metabolic homeostasis, but may not directly lead to overt diabetes in the early stage. With increased insulin secretion to compensate for insulin resistance, baseline blood glucose levels can be maintained within normal ranges, but the patients may demonstrate impaired responses to prandial carbohydrate loading and to oral glucose tolerance tests. The chronic over-stimulation of insulin secretion gradually diminishes and eventually exhausts the islet beta-cell reserve. A state of absolute insulin deficiency ensues and overt clinical diabetes becomes fully blown (DeFronzo 1988; Seely and Olefsky 1993). The transition of impaired glucose tolerance to type 2 diabetes can also be influenced by ethnicity, degree of obesity, distribution of body fat, sedentary lifestyle, aging, and other concomitant medical conditions (Clark 1998).

The quality of life of type 2 diabetic patients with chronic and severe hyperglycemia is adversely affected. Characteristic symptoms of tiredness and lethargy can become severe and lead to a decrease in work performance in adults and an increase of falls in the elderly (Davidson 1991). The most common acute complications are metabolic problems (hyperosmolar hyperglycemic nonketotic syndrome or HHNS) and infection. The long-term complications are macro-vascular complications (hypertension, dyslipidemia, myocardial infarction, stroke), micro-vascular complications (retinopathy, nephropathy, diabetic neuropathy, impaired cardiovascular reflexes, sexual dysfunction), and diabetic foot disorders. Type 2 diabetes is made up of different forms each of which is
characterized by a variable degree of insulin resistance and β-cell dysfunction and which together lead to hyperglycaemia (Wada 2001).

**1.3.2.1 Pathophysiology of Type 2 Diabetes**

Diabetes mellitus actually is a group of metabolic diseases characterized by hyperglycemia arising as a consequence of a relative or absolute deficiency of insulin secretion, resistance to insulin action, or both (Gerich 2003; Kahn 2003). Although diabetes mellitus is recognized by its characteristic hyperglycemia, the metabolic derangements are more pervasive, involving altered metabolism of carbohydrates, fats, and proteins. As a function of time and consequent to the metabolic disruption, diabetic patients may suffer the tragic ravages of long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Type 2 diabetes, the more common type, is usually due to resistance to insulin action in the setting of inadequate compensatory insulin secretory response (Gerich 2003; Kahn 2003). This is depicted in fig.1. Insulin resistance is actually quite common because it arises as a consequence of obesity, a sedentary lifestyle, and aging (fig.2), with resulting hyperglycemia and diabetes, blood pressure elevation, and dyslipidemia. In fact, collectively these abnormalities, which often occur together, have been designated the “metabolic syndrome” or more properly the “dysmetabolic syndrome.” Type 2 diabetes does not emerge in all persons with insulin resistance but rather only in those with a defect in insulin secretory capacity (presumably genetic) such that pancreatic insulin secretion fails to compensate for the insulin resistance.

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**Fig. 1:** Schematic depiction of the dual defect that is necessary for type 2 diabetes to be manifest: insulin resistance in the setting of impaired β-cell function inadequate to compensate for the insulin resistance.
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INTRODUCTION

Glucose intolerance
Type 2 diabetes
Dyslipidemia
high TG, low HDL
small dense LDL
Hypertension
Aging
Insulin Resistance
Genetics
Obesity
high W/H ratio
gluttony
Sedentary lifestyle
slothfulness

Fig. 2: Causes and consequences of insulin resistance. Insulin resistance arises from obesity (particularly central obesity), a sedentary lifestyle, and aging (perhaps related to progressive loss of muscle mass or sarcopenia) and may have a genetic proclivity to occurrence in some individuals. Potential consequences of insulin resistance include hyperglycemia and type 2 diabetes, blood pressure elevation (potentially leading to hypertension in those with a genetic risk of essential hypertension), and a dyslipidemia characterized by elevated triglycerides, low HDL cholesterol, and small, dense LDL cholesterol (an atherogenic lipid pattern).

1.3.2.2 Metabolic Staging of Type 2 Diabetes

Insulin resistance, the failure to respond to normal circulating concentrations of insulin, is a common state associated with obesity, aging, a sedentary lifestyle, as well as a genetic predisposition. The failure of insulin to stimulate glucose uptake in muscle appears to be a primary defect. Also in certain fat depots, subsequent resistance to the antilipolytic effects of insulin causes increased lipolysis and fatty acid release. These fatty acids attenuate the ability of insulin to suppress glucose production in the liver, but allow a continual increase in insulin-stimulated fatty acid synthesis. Thus, this dysregulation of carbohydrate and lipid metabolism accelerates the progression of insulin resistance. β-cells of the pancreas normally compensate for the insulin resistant state by increasing basal and postprandial insulin secretion, further aggravating insulin resistance. At some point, the β-cells can no longer compensate, failing to respond appropriately to glucose. This ultimately leads to the deterioration of glucose homeostasis and the development of glucose intolerance, the inability to properly dispose of glucose (fig.3). Every year, approximately 5 to 10% of glucose intolerant patients progress to frank diabetes. The liver produces more glucose in an unregulated fashion, and the β-cells undergo progressive
decompensation, resulting in the late stages of the disease, where high doses of exogenous insulin may be required (Maiztegui, Borelli et al. 2009).

Fig. 3: Metabolic Staging of Type 2 Diabetes; Type 2 diabetes is characterized by a progressive decrease in insulin action, followed by an inability of the β-cells to compensate for insulin resistance. Insulin resistance is the first abrasion, due to interactions among genes, aging and metabolic changes produced by obesity. Insulin resistance in visceral fat leads to increased fatty acid production, which exacerbates insulin resistance in liver and muscle. The β-cell compensates for insulin resistance by secreting more insulin. Ultimately, the β-cell can no longer compensate, leading to impaired glucose tolerance and diabetes

1.3.3 Complications of diabetes

1.3.3.1 Acute Complications

These include diabetic ketoacidosis and non ketotic hyperosmolar state. While first one is seen primarily in individuals of type 1 diabetes mellitus, the later is prevalent in individuals of type 2 diabetes mellitus. Both disorders are associated with absolute or relative insulin deficiency, volume depletion and altered mental state. In diabetic ketoacidosis, insulin deficiency is combined with counter regulatory hormone excess (glucagon, catecholamines, cortisol and growth hormone). The decreased ratio of insulin to glucagon promotes gluconeogenesis, glycogenolysis and ketone body formation in the
liver and also increases free fatty acids and amino acid delivery from fat and muscle to the liver. Ketosis results from marked increase in free fatty acids release from adipocytes due to increased lipolysis. In diabetic ketoacidosis, nausea and vomiting are often present. Cerebral oedema, an extremely serious complications are seen most frequently in children.

Nonketotic hyperosmolar state is most commonly seen in elderly individuals with type 2 diabetes mellitus. Its most prominent features include polyuria, orthostatic hypertension and a variety of neurologic symptoms including altered mental state, lethargy, obtundation, seizure and possibly coma.

1.3.3.2 Chronic complications

The chronic complications of diabetes mellitus affect many organ systems and are responsible for majority of morbidity and mortality. Chronic complications can be divided into vascular and non vascular complications. The vascular complications are further subdivided into microvascular (retinopathy, neuropathy and nephropathy) and macrovascular complications (coronary artery disease, peripheral vascular disease and cerebrovascular disease). Nonvascular complications include problems such as gastroparesis, sexual dysfunction and skin changes. As a consequence of its chronic complications, diabetes mellitus is the most common cause of adult blindness, variety of debilitating neuropathies, cardiac and cerebral disorders. The expenditure for treating complications of diabetes mellitus is more than controlling the disease.

Retinopathy

Diabetic retinopathy occurs in three fourth of the total affected diabetic subjects, having diabetes mellitus for more than 15 years and is the most common cause of blindness. There is appearance of retinal vascular lesions of increasing severity, culminating in the growth of new vessels. Diabetic retinopathy is classified into two stages namely non-proliferative and proliferative. Non-proliferative stage appears late in the first decade or early in the second decade of disease and is marked by retinal vascular microaneurysms, blot haemorrhages and cotton wool spots including loss of retinal pericytes, increased retinal vascular permeability, alterations in regional blood flow and abnormal retinal microvasculature, all of which lead to retinal ischemia. In proliferative retinopathy, there is appearance of neovascularisation in response to retinal hypoxia. The newly formed vessels may appear at the optic nerve and/or macula and rupture easily leading to vitreous haemorrhage, fibrosis and ultimately retinal detachment (Aiello, Rand et al. 1981).

Neuropathy
About half of all people affected with diabetes have some degree of neuropathy, which can be polyneuropathy, mono-neuropathy and/or autonomic neuropathy. In polyneuropathy there is loss of peripheral sensation, which when coupled with impaired microvascular and macro vascular junction in periphery can contribute to non healing ulcers, the leading cause of non traumatic amputation. There is thickening of axons, decrease in microfilaments and capillary narrowing involving small myelinated or non myelinated C-fibres. It can occur both from direct hyperglycemia induced damage to the nerve parenchyma and from neuronal ischemia leading to abnormalities of micro vessels such as endothelial cell activation, pericyte degeneration, basement membrane thickening and monocyte adhesion. Mono-neuropathy is less common than polyneuropathy and includes dysfunction of isolated cranial or peripheral nerves. Autonomic neuropathy can involve multiple systems including cardiovascular, gastrointestinal, genitourinary, sudomotor and metabolic systems (Chen, Wei et al. 1997).

**Nephropathy**

Nephropathy is the major cause of end stage renal disease. There are glomerular hemodynamic abnormalities resulting in glomerular hyperfiltration, leading to glomerular damage as evidenced by microalbuminurea. There is overt proteinuria, decreased glomerular filtration rate and end stage renal failure. Dysfunction of glomerular filtration apparatus is manifested by microalbuminurea and is attributed to changes in synthesis and catabolism of various glomerular basement membrane macromolecules such as collagen and proteoglycans leading to an increase in glomerular basement thickening. Another possible mechanism to explain the increase in permeability of the glomerulus is increase in the renal VEGF levels that are observed in preclinical models of diabetes, since VEGF is both, an angiogenic and permeability factor (Ritz, Ogata et al. 2000).
2 REVIEW OF LITERATURE

2.1 Experimental animal models for studying pathophysiology of type 2 diabetes mellitus and antidiabetic drug discovery

The pathogenesis of type 2 diabetes is complex involving progressive development of insulin resistance in liver and peripheral tissues accompanied by a defective insulin secretion from pancreatic beta cells leading to overt hyperglycaemia (Cheng 2005). In addition to genetic predisposition, the risk of developing type 2 diabetes in humans increases with age, obesity, cardiovascular disease (hypertension, dyslipidaemia) and a lack of physical activity (Ramarao and Kaul 1999; Cheng 2005). Generally, current therapeutic strategies for type 2 diabetes are limited and involve insulin and four main classes of oral antidiabetic agents that stimulate pancreatic insulin secretion (sulphonylureas and rapid-acting secretagogues/insulinotropics e.g., glibenclamide, glipizide, rapaglinide), reduce hepatic glucose production (biguanides e.g., metformin), delay digestion and absorption of intestinal carbohydrate (α-glucosidase inhibitors e.g., acarbose) or improve insulin action [thiazolidinediones (TZDs) e.g., pioglitazone, rosiglitazone]. Each of above agents suffers from generally inadequate efficacy and number of serious adverse effects. Thus, there are wide variety of newer therapeutic agents/strategies being examined for the treatment of type 2 diabetes, most of all currently under preclinical and early clinical stages of drug development (Ramarao and Kaul 1999; Krentz and Bailey 2005). Due to complex interaction among multiple susceptibility genes and between genetic and environmental factors, genetic analysis of diabetes is difficult and poorly understood in humans. Moreover, diabetes research in humans is impeded by obvious ethical considerations, because provocation of disease is strictly impermissible in man. Animal models of diabetes are therefore greatly useful and advantageous in biomedical studies because they offer promise of new insights into human diabetes. Inbred animal models, in which the genetic background is homogeneous and environmental factors can be controlled, are therefore valuable in genetic dissection of multifactorial diseases. Most of the available models are based on rodents because of their small size, short generation interval, easy availability and economic considerations; however, non-rodent models of diabetes are urgently needed as a valuable supplement to rodents for both practical and physiological reasons with respect to humans.

Although the symptoms of type 2 diabetes are well known, the genetic and physiological mechanisms causing these symptoms are poorly understood. Therefore, while the selection of appropriate experimental models to screen new antidiabetic drug is
critical, it is also difficult. One basic approach to drug discovery is to select specific cellular or molecular targets considered important in type 2 diabetes. An additional or alternative approach is to select \textit{in-vivo} models which have diabetic symptoms. In the drug discovery process, models are employed for a variety of purposes. Models are used to identify new compounds with no previous history of use for diabetes treatment (screening). Models are also used to understand the physiological effects, pharmacokinetics and toxicity of drugs or compounds in development (characterization). Finally, models can be used to further the understanding of disease mechanisms (basic research).

Animal models are essential to the drug discovery process in that all compounds intended for testing in human patients must first be tested in animals. The point in the drug discovery process at which a company initiates animal testing is largely a matter of strategy. Because \textit{in-vitro} models can be readily adapted to high throughput screening of large libraries of compounds, many company identify their initial ‘hits’ using \textit{in-vitro} models that represent specific cellular pathways or processes believed to be important in diabetes (Kubinyi 1995). While this approach dramatically increases the capacity of a screening programme, it also risks the possibility that ‘active’ lead compounds will not be active \textit{in-vivo}, or that the assay itself is not relevant to human diabetes. Some companies avoid this risk by screening test materials in animal models, although this approach generally reduces screening capacity. In order to reduce this risk, some companies narrow the range of materials tested by using compounds of a specific chemical class (Maxwell 1984) or materials with a history of medicinal usage (Oubre, Roy et al. 2007).

In addition, some animal models are better adapted than others for detecting specific types of antidiabetic activity. Anti-hyperglycaemic compounds such as metformin may not be detectable in normoglycaemic animal models, while insulin secretagogues and insulin sensitizers may require a model with intact insulin secretory response in order to show efficacy. Another consideration in an industrial research environment is the practicality of the animal model selected. For example, screening and medicinal chemistry campaigns generally require the testing of many compounds, but little detailed characterization of each compound. In these situations, using smaller animals, such as mice, will reduce the expense of producing test materials. Advanced efficacy studies or toxicological examinations which require invasive procedures or large blood and tissue samples may be facilitated by using animals with a large body size, such as rats.
2.1.1 Animal models of type 2 diabetes and their classification

Animals exhibiting a syndrome of insulin resistance and type 2 diabetes, with characteristics similar to humans, comprise a wide range of species with genetic, experimental or nutritional causation. Some animals with inherent diabetes have pancreas with ‘sturdy’ beta cells capable of maintaining robust, lifelong insulin secreting capacity characterized by severe hyperinsulinaemia with only mild to moderate hyperglycaemia throughout the life e.g., Zucker fatty rats (ZFR), \(ob/ob\) (obese), KK mouse and (corpulent) \(cp\) rat group. At the other end of spectrum, some species possess ‘brittle or labile’ pancreatic beta cells allowing only for transient insulin hypersecretion with short-term obesity (Kulkarni and Zisman 2003).

**Table:** Classification of type 2 diabetes in animals

<table>
<thead>
<tr>
<th><strong>Model category</strong></th>
<th><strong>Type 2 diabetic models</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous or genetically derived diabetic animals</td>
<td>(ob/ob) mouse</td>
</tr>
<tr>
<td></td>
<td>(db/db) mouse</td>
</tr>
<tr>
<td></td>
<td>KK mouse</td>
</tr>
<tr>
<td></td>
<td>Zucker fatty rat</td>
</tr>
<tr>
<td></td>
<td>Obese rhesus monkey</td>
</tr>
<tr>
<td>Diet/nutrition induced diabetic animals</td>
<td>Sand rat,</td>
</tr>
<tr>
<td></td>
<td>C57/BL 6J mouse</td>
</tr>
<tr>
<td></td>
<td>Spiny mouse</td>
</tr>
<tr>
<td>Chemically induced diabetic animals</td>
<td>Low dose AXN adult rats, mice and rabbit</td>
</tr>
<tr>
<td></td>
<td>STZ adult rats, mice</td>
</tr>
<tr>
<td></td>
<td>Neonatal STZ rat</td>
</tr>
<tr>
<td>Surgical diabetic animals</td>
<td>VMH lesioned dietary obese diabetic rat</td>
</tr>
<tr>
<td></td>
<td>Partial pancreatectomized animals e.g. dog, primate, pig &amp; rats</td>
</tr>
<tr>
<td>Transgenic/knock-out diabetic animals</td>
<td>(\beta_3) receptor knockout mouse</td>
</tr>
<tr>
<td></td>
<td>Uncoupling protein (UCP1) knock-out mouse</td>
</tr>
<tr>
<td></td>
<td>Transgenic or knock out mice of insulin signalling proteins e.g. IRS-1, IRS-2, GLUT-4, PTP-1B and others PPAR-g tissue specific knockout mouse</td>
</tr>
<tr>
<td></td>
<td>Glucokinase or GLUT 2 gene knockout mice</td>
</tr>
</tbody>
</table>
Further, the large number of new animal models developed in the recent years by genetic engineering/molecular biological techniques including transgenic and knock-out mice has initiated a new era in diabetes research and are useful for the investigation of aetiopathogenesis of diabetes and for testing various new chemical entities (NCEs) for the treatment of diabetes. Further, the expression and severity of metabolic, hormonal and pathologic abnormalities of diabetes perhaps varies in different animals according to the genetic background, nutrition, age, sex, species and even strain. Generally, the animal models of diabetes exhibit similar characteristic features such as chronic hyperglycaemia, hyper or normo or hypoinsulinaemia while the symptoms of clinical diabetes viz., polyuria, polydypsia, polyphagia, lethargy may also appear in several models of diabetes. Apart from the defects in glucose metabolism, the altered lipid metabolism associated with increase in plasma lipid levels is commonly observed in many animal models as in human diabetic patients. In addition, they exhibit various diabetic complications such as neuropathy, nephropathy, cardiomyopathy, atherosclerosis, hypertension and various others (Velasquez, Kimmel et al. 1990; McNeil 1999; Shafrir, Porte et al. 2003).

2.1.1.1 Spontaneous type 2 diabetic models

Spontaneously diabetic animals with type 2 diabetes may be obtained from the animals with one or several genetic mutations transmitted from generation to generation (e.g., ob/ob, db/db mice) or be selected from non-diabetic outbred animals by repeated breeding over several generations [e.g., (GK) rat, Tsumara Suzuki Obese Diabetes (TSOD) mouse]. These animals generally inherited diabetes either as single or multigene defects. The metabolic peculiarities result from single gene defect (monogenic) which may be due to dominant gene (e.g., Yellow obese or KK/Ay mouse) or recessive gene (diabetic or db/db mouse, Zucker fatty rat) or it can be of polygenic origin [e.g., Kuo Kondo (KK) mouse, New Zealand obese (NZO) mouse] (Ktorza, Bernard et al. 1997). Type 2 diabetes occurring in the majority of humans is a result of interaction between environmental and multiple gene defects, though certain subtypes of diabetes also exist with well defined cause [i.e., maturity onset diabetes of youth (MODY) due to defect in glucokinase gene] and this single gene defects may cause type 2 diabetes only in few cases. Therefore, polygenic animals represent the human condition more closely when compared to monogenic animals (McIntosh and Pederson 1999).

*ob/ob mouse*

*ob/ob* mouse (obese mouse) (now relabeled as Lep<sup>ob</sup>) is inherited as (monogenic) autosomal recessive mutation on chromosome 6 (obese) in C57BL/6J mouse strain,
originating from the Bar Harbor, Jackson laboratory (Shafrir, Porte et al. 2003). The mutation in \( ob/ob \) mice is now identified to occur in leptin gene, which encodes for leptin. Homozygous mutant mice exhibit rapid gain in body weight and may reach up to three times the normal weight of wild type control. There is impaired thermogenesis detectable very early at 10 days. In addition, there is marked hyperphagia and decreased energy expenditure resulting in increased carcass lipid with obvious obesity by approximately 4 weeks. Along with obesity, it also exhibits diabetes-like syndrome manifested as hyperglycaemia; mild impaired glucose tolerance, severe hyperinsulinaemia, sub fertility and impaired wound healing. Hyperglycaemia is only transient (subsiding around 14 to 16 wk). However, when \( ob \) gene is expressed mice become severely diabetic with regression of islets and early death (Bell and Hye 1983; Shafrir, Porte et al. 2003). The obesity is characterized by both hypertrophy and hyperplasia of pancreatic islets (Velasquez, Kimmel et al. 1990). Hyperinsulinaemia does not develop until after the increase in body weight and probably results show it. Insulin resistance is associated with hepatic glucose overproduction, increased activity of gluconeogenic enzymes, decreased activity of glycolytic and glycogen synthesis enzymes and increased lipogenesis in the liver. The circulating glucose concentration is almost maintained normally in adult C57BL/6J (\( ob/ob \)) mouse due to sustained hyperinsulinaemia. At molecular level, insulin resistance in \( ob/ob \) mice appears to be due to diminished insulin binding to receptors, impaired insulin receptor (IR) autophosphorylation, and reduced signal transduction (McNeil 1999). Since this model is severely obese, hyperinsulinaemic and insulin resistant throughout its life, the agents that decrease the body weight and improves peripheral insulin sensitivity like insulin sensitizers, antiobesity and some other antihyperglycaemic agents have been largely tested and shown to produce antidiabetic activity (Himms-Hagen and Danforth 1996; Zhang, Salituro et al. 1999).

**db/db mouse**

The \( db/db \) (diabetic) mouse (now relabeled as \( lepr^{db} \)) is originally derived from an autosomal recessive mutation on chromosome 4 in mice of C57BL/KsJ strain originating from Bar Harbor, Maine (Shafrir, Porte et al. 2003). The mutation in this diabetic animal was traced to \( db \) gene, which encodes for the leptin receptors. These mice are spontaneously hyperphagic insulin oversecretors becoming obese, hyperglycaemic, hyperinsulinaemic and insulin resistant within first month of age and develop hypoinsulinaemia, hyperglycaemia later with a peak between 3-4 months of age. Animals then exhibit ketosis, progressive body weight loss and do not survive longer than 8-10
months. In \textit{db/db} mice, lack of leptin receptors result in similar hypothalamic disturbances and neuropeptide Y abnormalities as in \textit{ob/ob} mice, but the leptin is still produced and NPY induced hyperinsulinaemia plus hypercorticosteronism result in \textit{ob} gene hyperexpression and hyperleptinaemia. Unlike \textit{ob/ob} mice, exogenous administration of leptin fails to elicit effect on food intake and body weight as there is defect in leptin receptor (McNeil 1999). \textit{db/db} mice have been commonly and extensively used for the investigation of type 2 diabetes/diabetic dyslipidaemia and screening of variety of agents such as insulin mimetic and insulin sensitizers (Zhang, Salituro et al. 1999; Nuss and Wagman 2000; Knouff, Briand et al. 2004).

\textit{Zucker diabetic fatty rat}

It is a substrain of ZFR selectively inbred for hyperglycaemia and is highly useful for investigating the mechanism of type 2 diabetes. Unlike ZFR, male Zucker diabetic fatty (ZDF) rat progresses to frank diabetes due to failure to compensate adequately for insulin resistance. It is less obese but more insulin resistant than ZFR (Shafrir 1992). Males are more prone to the development of diabetes usually at 7-10 wk after birth. This model is presently available with Charles River laboratories, Indianapolis, IN, USA. Inspite of being obese and insulin resistant the littermates do not develop diabetes and serve as control. In contrast to \textit{fa/fa} rats, the ability to over secrete insulin to compensate peripheral insulin resistance is limited, and the beta cells are brittle, easily succumbing to the pressure of over-secretion. Studies on cell turnover in these animals suggest that the primary defect lies not in an ability of beta cells to proliferate but rather in an enhanced rate of apoptosis (Pick, Clark et al. 1998).The model exhibits impaired insulin secretory beta cell response to glucose while it remains intact to non glucose secretagogues like arginine, a phenomenon similar to human type 2 diabetic patients. Down regulation of beta cell GLUT-2 transporters coupled with impaired insulin synthesis has been reported to be responsible for hyperglycaemia in ZDF rats. Decreased glucose transport activity associated with decreased GLUT-4 levels is observed in adipose tissue and skeletal muscles of ZDF rats. The previous study demonstrated the deleterious phenomenon of lipotoxicity of high plasma free fatty acid and beta cell triglyceride levels on beta cell function in ZDF rats (Shafrir and Ziv 1998). ZDF rat has been most commonly used for investigating the mechanisms associated with insulin resistance and beta cell dysfunction in type 2 diabetes, as well as for testing insulin sensitizers, insulinotropic and various other agents (Himms-Hagen and Danforth 1996; Zhang and Moller 2000; Nielsen 2005).

\textit{Fructose-induced type-2 diabetes and insulin resistance}
Critical insights into the etiology of insulin resistance have been gained by the use of animal models where insulin action has been modulated by strictly controlled diet, an intervention not possible in human studies. Early work clearly demonstrated that diets high in simple sugars (in particular fructose) led to insulin resistance (Storlien, Higgins et al. 2000). Chronic administration of fructose to normal rats led to both hyperinsulinemia and in-vivo insulin resistance. The insulin resistance resulting from chronic feeding is due to the diminished ability of insulin to suppress hepatic glucose production, and not to a decrease in insulin-stimulated glucose uptake by muscles. Insulin resistance, hyperinsulinemia and hypertriglyceridemia have been demonstrated in Sprague-Dawley rats fed with fructose-enriched diet (Laville and Nazare 2009). Although the exact molecular mechanism involved is unknown, it is believed that chronic feeding of fructose affects the early steps of insulin action in muscle and liver. This conclusion was drawn by comparing the phosphorylation status of the insulin receptor (IR) and insulin receptor substrate-1 (IRS-1), as well as the association of the IRS-1 with phosphotidylinositol 3-kinase (PI-3Kinase), and phosphotyrosine phosphatase (SHP2) in the liver and muscle. There were no differences in IR and the IRS-1 protein level in the liver and muscles of rats in control and fructose fed group. However, tyrosine-phosphorylation of the insulin receptor after insulin stimulation was reduced significantly in the liver of fructose fed rats. These data suggest that changes in the early steps of insulin signal transduction may have an important role in the insulin resistance observed in these rats (Suzuki, Nanda et al. 2008).

C57BL/6J mouse

Type 2 diabetic model was initially developed in Japan by simply feeding high fat feed to non obese, non diabetic C57BL/6J mouse strain and is now available at Jackson laboratory, Bar Harbor (Shafrir, Porte et al. 2003). It is characterized by marked obesity, hyperinsulinaemia, insulin resistance and glucose intolerance (Stewart, Wang et al. 2009). In addition, the mice exhibit marked fasting as well as basal hyperglycaemia in contrast to normal basal glucose level seen in C57BL/6J (ob/ob) mice. These mice are demonstrated to develop peripheral leptin resistance. They manifest most of the characteristic features of the patients with genetic predisposition to develop type 2 diabetes when they become obese. This animal model represents both genetic and environmental risk factors in contrast to C57BL/6J (ob/ob) mouse in which onset of symptoms is highly genetically determined. Further, its usefulness for drug testing has been reported in the literature as these mice treated with orally active inhibitor of dipeptidyl peptidase-IV are shown to
SECTION-A  | REVIEW OF LITERATURE: Animal models for diabetes

have normalized glucose tolerance in association with augmented insulin secretion (Gupta, Walunj et al. 2009).

2.1.1.2 Chemically induced type 2 diabetic models

Chemically induced models of diabetes are common in elucidating the possible role of environmental factors involved in the endocrine pancreatic destructive processes and subsequent development of diabetes.

*Alloxan-induced diabetic animals*

Since the initial findings in 1943 of alloxan induced beta cell necrosis in rabbits, this compound has long been used for inducing experimental diabetes. Alloxan is a uric acid derivative and is highly unstable in water at neutral pH, but reasonably stable at pH 3. Alloxan acts by selectively destroying the pancreatic beta islets leading to insulin deficiency, hyperglycaemia and ketosis (Rerup 1970). Alloxan causes diabetes in many rodent and non rodent animals and is most preferably used in case of rabbit because of the relative ineffectiveness of streptozotocin (STZ) in rabbits for induction of diabetes and development of well characterized diabetic complications (Rerup 1970). However, guinea pig and recently musk shrew have been reported to be resistant to the action of alloxan due to certain unclear mechanisms (Ohno, Kitoh et al. 1998). Because of its low stability, very short half-life (less than 1 min) and acidic nature of solution, intravenous route of administration of alloxan is preferred. The hypoglycaemic phase may be quite severe and therefore alloxan should not be given to fasted animals. The alloxan treated animals exhibit severe hyperglycaemia, glucosuria, hyperlipidaemia, polyphagia, polydypsia and other symptoms of uncontrolled diabetes and also develop various complications such as neuropathy, cardiomyopathy, well marked retinopathy and others. Alloxan is disadvantageous as the percentage incidence of diabetes is quite variable and is not proportionately related to increasing doses of alloxan (Battell, Yuen et al. 1999). Further, the incidence of ketosis and resulting mortality is high. The reversal of hyperglycaemia due to pancreatic regeneration is early and common in case of alloxan treated animals. Because of these limitations, alloxan is now less preferred over STZ for induction of diabetes in laboratory animals.

*Streptozotocin (STZ)-induced diabetic animals*

Streptozotocin is an antibiotic derived from *Streptomyces achromogenes* and structurally is a glucosamine derivative of nitrosourea. Rakieten and his associates first demonstrated the diabetogenic property of STZ in dogs and rats in 1963. Like alloxan, it causes hyperglycaemia mainly by its direct cytotoxic action on the pancreatic beta cells.
The evidences are accumulating on the mechanisms associated with diabetogenecity of STZ. Its nitrosourea moiety is responsible for beta cell toxicity, while deoxyglucose moiety facilitates transport across the cell membrane. Like alloxan, the involvement of free radical generation and resulting alteration of endogenous scavengers of these reactive species have been reported in STZ diabetogenecity. Recently, a animal model of type 2 diabetes has been produced by combination of STZ and NAD administration in adult rats (Masiello, Broca et al. 1998). The rats administered NAD (230 mg/kg, i.p.) 15 min before STZ (65 mg/kg, i.v.) has been shown to develop moderate and stable non-fasting hyperglycaemia without any significant change in plasma insulin level. As NAD is an antioxidant which exerts protective effect on the cytotoxic action of STZ by scavenging free radical, its co-administration with STZ results only in minor damage to pancreatic beta cell mass producing type 2 diabetes. Therefore, this model is found to be an advantageous tool for investigation of insulinotropic agents in the treatment of type 2 diabetes.

**Neonatal STZ-induced model of Type 2 diabetes**

Animal studies have been useful in exploring the molecular mechanisms underlying the development of type 2 diabetes. Neonatal rats treated with STZ (80-120 mg/kg) at birth (nO) or within the first 5 days following birth (nI-5) experience severe pancreatic β-cell destruction, accompanied by a decrease in pancreatic insulin stores and a rise in plasma glucose levels (Weir and Bonner-Weir 1981; Blondel, Bailbe et al. 1989). However, in contrast to adult rats treated with STZ, the β-cells of the treated neonates partially regenerate (Wang, Bouwens et al. 1996). Following an initial spike in plasma glucose, the STZ-treated neonatal rat become normoglycaemic by 3 weeks of age. In the next few weeks, the β-cell number increases, the extent depending upon both the age at which the animal is treated with STZ and the species of the treated rat (Bonner-Weir, Trent et al. 1981; Weir and Bonner-Weir 1981; Blondel, Bailbe et al. 1989; Iwase, Nunoii et al. 1994; Wang, Bouwens et al. 1996). The aging process also affects the diabetic state of the STZ-treated rat. Although 10 weeks old STZ-Wistar rats exhibit normal non-fasting glucose and insulin level, they are markedly glucose intolerant. Moreover, these animals become progressively more glucose intolerant with age. By 6 months of age, a glucose challenge provokes a condition of severe hyperglycaemia and hyperinsulinemia (Schaffer and Wilson 1993).
2.1.1.3 Transgenic and knockout type 2 diabetic models

The nature of marked heterogeneity with multifactorial genetic and environmental background of diabetes poses challenges to identify exact molecular mechanisms involved in treatment of diabetes. Recently, transgenic technique is gaining momentum as it provides excellent opportunity for investigation of role of specific gene products and its mechanisms probably involved in disease conditions under its own physiological (as opposed to in-vitro) environmental conditions. Transgenic animals are generally helpful in giving insights into gene regulation and development, pathogenesis and finding new targets and the treatment of disease. In general, transgenic animals particularly mice are usually created by transferring and altering the site or level of expression of functional gene (transgene) or by deleting specific endogenous genes (knockout) or placing them under the control of alternate promoter regions (Livingston 1999).

The transgenic and knockout models are developed for studying the role of genes and their effects on peripheral insulin action such as insulin receptor, IRS-1, IRS-2, glucose transporter (GLUT-4), peroxisome proliferator-activated receptor-γ (PPAR-γ) and tumour necrosis factor-α (TNF-α) as well as in insulin secretion such as GLUT-2, glucokinase (GK), islet amyloid polypeptide (IAPP) and GLP-1 and in hepatic glucose production (expression of PEPCK) associated with development of type 2 diabetes. Further, combination or double knockout mouse models including defect in insulin action and insulin secretion (e.g., IRS-1⁻/⁻/GK⁺⁺ double knockout) have been produced which clearly illustrate the mechanisms associated with development of insulin resistance and β-cell dysfunction leading to overt hyperglycaemic state in human type 2 diabetes. These above genetically modified animals exhibit various phenotypic features of type 2 diabetes varying from mild to severe hyperglycaemia, insulin resistance, hyperinsulinaemia, impaired glucose tolerance and others as explained in detail elsewhere (Shafrir 1992; Nandi, Kitamura et al. 2004; Gray, Nora et al. 2005; Plum, Wunderlich et al. 2005). During the last 5 years, tissue specific knockout mouse models have been achieved, allowing further insight into the insulin action with respect to particular target tissues (muscle, adipose tissue and liver) associated with insulin resistance and type 2 diabetes (Nandi, Kitamura et al. 2004; Gray, Nora et al. 2005; Plum, Wunderlich et al. 2005). Transgenic/knockout animals are currently used mostly for the mechanistic study in diabetes research and not usually recommended for screening programme as they are costly and difficult to generate.
2.2 Conventional oral antidiabetic agents

The general consensus on treatment of type 2 diabetes is that lifestyle management is at the forefront of therapy options. In addition to exercise, weight control, and medical nutrition therapy, oral glucose-lowering drugs and injections of insulin are the conventional therapies. Since the most important pathological process during the development of diabetes involves three key organs, i.e., pancreatic islets, liver, and skeletal muscle, almost all anti-diabetic therapies are aimed at these organs. Pharmacological treatment is indicated when fasting glucose level exceeds 140 mg/dl, the postprandial glucose level exceeds 160 mg/dl or HbA1c exceeds 8.0 percent (DeFronzo 1999).

In the United States, five classes of oral agents are approved for the treatment of type 2 diabetes. By conventional standards, oral therapy is indicated in any patient with type 2 diabetes in whom diet and exercise fail to achieve acceptable glycemic control (DeFronzo 1999). Although initial responses may be good, oral hypoglycemic drugs may lose their effectiveness in a significant percentage of patients. The drug categories include sulfonylureas, biguanides, alpha-glucosidase inhibitors, thiazolidinediones, and meglitinides.

2.2.1 Insulin Secretagogues

**Sulfonylureas (SUs),**

Sulfonylureas including first generation (e.g., tolbutamide) and second generation (e.g., glyburide) sulfonylureas, enhance insulin secretion from the pancreatic beta-cells. A significant side effect is hypoglycemia. Sulfonylurea therapy is also usually associated with weight gain due to hyperinsulinemia (Kelly 1995; Bodmer, Meier et al. 2008), which has been implicated as a cause of secondary drug failure.

**Meglitinide,**

Repaglinide is the first drug of the meglitinide class, a member of carbamoylmethyl benzoic acid family (glinides). This is structurally different from SUs.
but shows resemblance with glyburide. Recent addition to this class of compounds is nateglinide. Meglitinides stimulate the secretion of insulin from pancreatic β-cells. However, the action is mediated through a different binding site compared to the SU receptor of the β-cells and the drugs have different characteristics than those of SUs (Campbell and White 2008). Among potential advantages, these agents can reduce the postprandial glucose level to a higher degree and have a decreased risk of hypoglycaemia that is reversible when discontinued. Acarbose therapy is linked to elevation in serum transaminase levels and use of this agent is contraindicated in patients with liver cirrhosis. Similarly, concentrations of AGIs have been shown to increase the degree of renal dysfunction proportionally and their use in patients with serum creatinine level >2.0 mg/dl is not recommended. However, the long-term implications of the use of this class of drugs on chronic complications have not been examined. Because of their quick onset of action doses are prescribed just before meals. Whenever the meal is omitted throughout the day, the doctors advise the patients to skip the drug too. Similarly, for an extra meal, extra dose of the drug is required. Repaglinide has a better pharmacokinetics profile (rapid absorption, short duration of action) and offers good long-term glycaemic control combined with a low risk of severe hypoglycaemia.

![Fig. Insulin Secretagogues; repaglinide and nateglinide](image)

2.2.2 Biguanides

Metformin, phenformin and buformin were introduced in the market shortly after SUs. Buformin was introduced in a limited manner but the other two were widely used till 1977 when phenformin was withdrawn in many countries due to reported cases of lactic acidosis. Metformin is currently the only drug in this antidiabetic class available in US, where it has attained the top-selling oral hypolipidemic agent position. It works by reducing hepatic glucose output (HGO) through inhibition of gluconeogenesis (Kim, Park et al. 2008) and to a lesser extent, enhancing insulin sensitivity in hepatic and peripheral tissues. Other effects include decreased appetite and food absorption and reduction in LDL-cholesterol levels. Recent report Zhou et al., (2001) indicates that effects of metformin may be mediated at least in part via Adenosine Monophosphate-activated

TH-17166
Protein Kinase (AMPK) activation. Interestingly, metformin has been found to lower the body weight unlike other antidiabetic drugs (Golay 2008).

In general, metformin does not show any hypoglycemia. It is a stable compound, does not bind to plasma proteins and is excreted unchanged through urine. The disadvantage of this drug is that due to its low bioavailability clinical dose is very high. During metformin treatment 20% of patients suffer from diarrhea, abdominal discomfort, nausea, metallic taste and anorexia (Kecskemeti, Bagi et al. 2002). The biguanides, phenformin and metformin, are associated with an increased incidence of lethal lactic acidosis (Finkle 2009). Several attempts have failed to get a better biguanide than metformin.

![Phenformin, Buformin, Metformin](image)

Fig. Biguanides; phenformine, buformin and metformin

2.2.3 Absorption Inhibitors

α-Glucosidase inhibitors (AGIs) reduce intestinal absorption of dextrin and disaccharides by inhibiting the action of brush border enzyme α-glucosidase in the small intestine, which degrade more complex carbohydrates into sugars. The AGI is a class of drug that does not target specific pathophysiological defects in type 2 DM. Acarbose is a competitive inhibitor of α-glucosidase. The other agent available in the market is miglitol. These agents delay the degradation of carbohydrates as well as retard the absorption of meal-derived glucose into circulation. Therefore, the largest impact of these drugs is on postprandial hyperglycaemia. The advantage of the AGIs is that they are not associated with hypoglycaemia and weight gain. The most alarming side effects associated with these drugs are at gastrointestinal level including abdominal discomfort, bloating, flatulence and diarrhea that are reversible when the drug discontinued (Ripsin, Kang et al. 2009).

![Acarbose, Miglitol](image)

Fig. Absorption Inhibitors; acarbose and miglitol
2.2.4 Insulin Sensitizers

Insulin resistance is the underlying factor that occurs in a vast majority of Type 2 diabetes and is a result of a decrease in sensitivity and/or insulin responsiveness in target organs. Insulin resistance leads to impairment in glucose uptake and storage, lipid metabolism and endothelium dysfunction. This post-receptor defect present in liver, fat and skeletal muscle is central to the pathobiology of syndrome X and type 2 diabetes. In most of the cases insulin resistance develops before hyperglycaemia. Several years of research in pharmaceutical companies worldwide recently led to the discovery of novel drugs targeted for sensitization of peripheral tissues to insulin (e.g. Rosiglitazone and pioglitazone).

**Thiazolidinediones**

Thiazolidinediones are represented by troglitazone, rosiglitazone and pioglitazone. These expensive oral agents work by improving insulin sensitivity in muscle and, to a much lesser extent, in the liver. These drugs decrease plasma triglyceride levels, but such decrease may be associated with weight gain and an increase in LDL-cholesterol levels. Liver toxicity is a concern requiring monthly monitoring of liver function. Since troglitazone is more toxic to the liver than rosiglitazone and pioglitazone (having resulted in dozens of deaths from liver failure), in March 2000 the FDA asked the manufacturer of troglitazone to remove the product from the market (Scheen 2008).

![Fig. Insulin sensitizers; troglitazone, pioglitazone and rosiglitazone](image)

2.2.5 Insulin therapy

The purpose of therapy in diabetes mellitus is to restore metabolism to normal, avoid symptoms due to hyperglycemia and glucosuria, prevent short term complications (infections, ketoacidosis etc.) and long term sequelae (Cardiovascular, retinal, neurological, renal etc.) Insulin ineffective in all forms of diabetes mellitus and is must for
type 1 cases. Many type 2 cases can be controlled by diet reduction in body weight and appropriate exercise. Insulin is needed by such cases when:

- Not controlled by diet and exercise or when these are not practicable.
- Primary or secondary failure of oral hypoglycaemics or when these drugs are not tolerated.
- Under weight patients
- Temporarily to tide over infections, trauma, surgery, pregnancy. In the preoperative period and during labour, monitored i.v. insulin infusions preferable.
- Any complication of diabetes e.g. Ketoacidosis, gangrene of extremities.

2.2.6 Insulin analogues

1. **Rapid acting (e.g. Lyspro, Aspart):** It is useful in children and in situations where other methods have failed to control post-prandial hyperglycemia.

2. **Long acting (Glargine):** it is used to treat type 1 diabetes. It is also used to treat people with type 2 diabetes, who need long-acting insulin to control their diabetes.
2.3 Alternative therapies for type 2 diabetes

Plant-based medicinal products have been known since ancient times, and several medicinal plants and their products have been used to control diabetes in the traditional medicinal systems of many cultures worldwide, including those of the Asian Indians, Chinese and South Americans. A limited number of these plant species have been studied and validated for their hypoglycaemic properties using diabetic animal models and in clinical studies using human subjects. In the recent days plant-based herbal drugs or botanicals are emerging as the primary components of holistic approaches to diabetes management (Mentreddy 2007).

Many conventional drugs have been derived from prototypic molecules in medicinal plants. Metformin exemplifies an efficacious oral glucose-lowering agent. Its development was based on the use of Galega officinalis to treat diabetes. To date, over 400 traditional plant treatments for diabetes have been reported, although only a small number of these have received scientific and medical evaluation to assess their efficacy. The hypoglycemic effect of some herbal extracts has been confirmed in human and animal models of type 2 diabetes. The World Health Organization Expert Committee on diabetes has recommended that traditional medicinal herbs be further investigated (Bailey and Day 1989). The following is a summary of several of the most studied and commonly used medicinal herbs (Gupta, Bajpai et al. 2008).

2.3.1 Aegle marmelos: Hindi name (Bel)

The trees grow throughout deciduous forest of India and ripen fruits are commonly used for delicacy. Aegle marmelos is widely used in Indian Ayurvedic medicine for the treatment of diabetes mellitus (Kamalakkanan and Prince 2003). Hypoglycemic effect of root bark decoction has been observed in normal fasted rats (Karunanayake, Welihinda et al. 1984). Leaf extract produced anti-hyperglycemic activity in alloxan diabetic rats along with decreased cholesterol and blood urea (Ponnachan, Paulose et al. 1993). In diabetic rats leaf extract exhibited insulin like activity (Paulose, Ponnachan et al. 1993). Aqueous leaf extract has been shown to improve the functional state of pancreatic cells in streptozotocin induced diabetic rats (Das, Padayathi et al. 1996). Anti-hyperglycemic activity caused by leaf extract in glucose fed hyperglycemic rats (Sachdewa, Raina et al. 2001). Aqueous fruit extract produces anti-hyperglycemic effect along with decreasing glycosylated haemoglobin level in STZ induced diabetic albino wistar rats (Kamalakkanan and Prince 2003). Hypoglycemic and antioxidant activity of leaves have been observed in
diabetic male albino rats (Upadhya, Shanbhag et al. 2004). Fruit extract of this plant also produced anti-diabetic, anti-hyperlipidaemic and antioxidant activity in STZ diabetic rats along with partial repair of damaged pancreatic islets (Kamalakkanan and Prince 2005). Treatment of severely diabetic rats for 14 days with aqueous extract of *Aegle marmelos* seeds reduced the fasting blood glucose by 60.84% and urine sugar by 75% than their pretreatment levels (Kesari, Gupta et al. 2006).

2.3.2 *Aloe vera: Hindi & common name (Aloe)*

The herbs are cultivated throughout India for its variety of medicinal properties. Dry sap of plant produced prominent anti-hyperglycemic response in type 2 diabetic patients, and in alloxan induced diabetic Swiss albino mice (Ghannam, Kingston et al. 1986). In a clinical study, it was reported that oral administration of aloe might be a useful adjuvant for lowering of blood glucose in diabetic patients (Vogler and Ernst 1999). *Aloe vera* leaf pulp extract showed hypoglycemic activity in type 1 and type 2 diabetic rats, the effect being enhanced in type 2 diabetes as compared with glibenclamide (Okyar, Can et al. 2001). Plant extract produced hypoglycemic activity along with controlled carbohydrate metabolizing enzymes in normal fasted, oral glucose fed and STZ-diabetic rats (Rajasekaran, Sivagnanam et al. 2004). Oral administration of ethanolic extract to STZ-diabetic rats for 21 days resulted in a prominent reduction of fasting blood glucose along with improved plasma insulin level of diabetic rats (Rajasekaran, Sivagnanam et al. 2005). Administration of the five phytosterols from *Aloe vera* namely, lophenol, 24-methyllophenol, 24-ethyl-lophenol, cycloartanol and 24-methylene-cycloartanol to severe type 2 diabetic mice for 28 days decreased the fasting blood glucose levels in diabetic rats (Tanaka, Misawa et al. 2006). Oral administration of *Aloe vera* gel extract to STZ-diabetic rats resulted in a significant reduction of fasting blood glucose and improved the plasma insulin level (Rajasekaran, Ravi et al. 2006).

2.3.3 *Andrographis paniculata: Hindi name (Kalmegha)*

This is an annual herb that grows throughout India. Plant extract effectively produced anti-hyperglycemic activity in normal rats (Borhanuddin, Shamsuzzoha et al. 1994). Antihyperglycemic and antioxidant activity of plant extract, has also been reported in diabetic rats (Zhang and Tan 2000). The andrographolide from plant increases the glucose utilization and lowers plasma glucose in diabetic rats lacking insulin (Yu, Hung et al. 2003). Significant reduction in blood glucose level was observed when hyperglycemic rats treated with 50mg/kg body weight aqueous extract of *Andrographis paniculata*. This
effect enhanced when freeze-dried material used at a dose of 6.25 mg/kg body weight, it reduced 61.8% blood glucose level (Husen, Pihie et al. 2004). The anti-diabetic potentials of plant restored the impaired estrous cycle in alloxan-induced diabetic rats (Reyes, Bautista et al. 2006).

2.3.4 **Gymnema sylvestre:** Hindi name (Gudmar)

Plants are grown in tropical regions of India and used as household remedy for diabetes (Kar, Choudhary et al. 2003). Oral administration of a water soluble fraction G-54 isolated from *Gymnema sylvestre* administered to 27 type 2 diabetic patients reduced their insulin requirement, lowered the fasting blood sugar and glycosylated haemoglobin content (Shanmugasundaram, Rajeswari et al. 1990a). Two water soluble fractions (GS-3 and GS-4) obtained from leaves were found to double the pancreatic islets and β-cell numbers in diabetic rats (Shanmugasundaram, Gopinath et al. 1990b). Alcoholic leaf extract lowered maximum blood sugar in fasted, glucose fed and diabetic rats along with insulin released from pancreatic β-cells (Chatopadhyay, Medda et al. 1993). In rats the insulin secretion from islets of Langerhans and several pancreatic β-cell lines induced by alcoholic extract in absence of other stimulus (Persaud, Al-Majed et al. 1999). Gymnemic acid IV, isolated from leaf produced potent hypoglycemic effect in STZ-diabetic mice (Sugihara, Nojima et al. 2000). Leaf extract has been observed to produce anti-hyperglycemic (Gholap and Kar 2003) and hypoglycemic (Gholap and Kar 2004) effects of in corticosteroid-induced diabetes mellitus, without altered serum cortisol concentration. A polyherbal formulation containing aqueous extracts of *Gymnema sylvestre* produced prominent hypoglycemic activity in normal and diabetic rats at a dose of 100-500mg/kg/day, orally for, 6 hours and for long-term, 6 weeks studies (Mutalik, Chetana et al. 2005). Gymnemic acid IV isolated from the leaves has been observed to produce hypoglycemic, anti-hyperglycemic, glucose uptake inhibitory and gut glycosidase inhibitory effects (Kimura 2006).

2.3.5 **Momordica charantia:** Hindi name (Karela)

The plant is an annual climber grown mostly in tropical India and commonly use as vegetable (Saxena, Mukherjee et al. 2006). Charantin isolated from *Momordica charantia* has been resembled to insulin and lowered blood sugar level of rabbits (Lolitkar and Rao 1966). In a clinical study of type 1 and type 2 diabetic patients the polypeptide-p isolated from fruit, seeds and tissue exhibited hypoglycemic activity without any side effect. The subcutaneous injection of (0.5unit/kg) lowered the blood sugar in gerbils and
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Charantin obtained from *M. charantia* induced hypoglycemic effect (Ng, Wong et al. 1986a) and also stimulated the insulin release and blocked the formation of glucose in blood stream (Ng, Wong et al. 1986b). Hypoglycemic effect and delayed cataract development was reported in alloxan diabetic rats treated with fruit extract (Srivastava, Venkatakrishna-Bhatt et al. 1988). Ethanolic extract of *M. charantia* was produced hypoglycemic activity in normal and STZ-diabetic rats; this was occurred possibly due to inhibiting glucose-6-phosphatase and fructose-1,6-bisphosphatase in liver, and stimulating hepatic glucose-6-phosphate dehydrogenase activities (Shibib, Khan et al. 1993). Oleanolic acid and momordin from plant, produced antihyperglycemic effect by inhibiting glucose transport in intestine of rats (Matsuda, Li et al. 1988). Fruit aqueous extract, and exercise potentially lowered blood sugar of type 2 diabetic and hyperinsulinemic rats (Miura, Itoh et al. 2004). Seed aqueous extract produced prominent reduction in blood glucose, glycosylated hemoglobin, lactate dehydrogenase, glucose-6-phosphatase, fructose-1, 6-bisphosphatase and glycogen phosphorylase along with increased hemoglobin, glycogen content and hexokinase, glycogen synthase activity (Sekar, Mukherjee et al. 1987). Anti-diabetic properties of plant such as charantin, vicine and polypeptide-p have the potential to be a part of dietary supplement for patients of diabetes (Krawinkel and Keding 2006). From *M. charantia* the major compounds, 5β,19-epoxy-3β,25-dihydroxycucurbita-6,23 (E)-diene (4) and 3β-7β, 25dihydroxycucurbita-5, 23 (E)-dien-19-al (5) administered at a dose of 400mg/kg produced hypoglycemic effect in ddY mice strain (Harinantenaina, Tanaka et al. 2006).

### 2.3.6 Syzygium cumini: Hindi name (Jamun)

Plants grow in different parts of India. The ripe fruits are used as part of dietary component. Oral administration of fruit pulp induced hypoglycemic activity in normal and STZ-diabetic rats along with insulin released from β-cells (Achrekar, Kakliji et al. 1991). Seed powder provided good symptomatic relief to 30 patients of diabetes (type 2) and regulated blood sugar level (Kohli and Singh 1993). Increased activity of hexokinase and decreased activity of glucose-6-phosphatase in liver produced blood sugar lowering effect at oral administration of aqueous seed extract to alloxan diabetic rats (Prince, Menon et al. 1997). Aqueous seed extract has been observed to produce hypoglycemic and antioxidant activity, and increase in haemoglobin content in rats (Prince, Menon et al. 1998). Alcoholic seed extract injection (20mg, intraperitoneally) reduced the blood sugar level to 37.17% at 3 hour and 46.68% at 6 hour of administration in alloxan diabetic mice along
with enhanced insulin secretion (Purohit and Daradka 2000). Decreased plasma glucose concentrations in STZ-induced diabetic mice was observed at oral administration of fruit extract (Grover, Yadav et al. 2002). Blood sugar lowering, hypolipidemic activity, increased serum insulin, increased glycogen content of liver and muscles and a fall in glycosylated haemoglobin level produced by ethanolic extract of seed (Sharma, Nasir et al. 2003). Ethanolic seed kernels extract of *S. cumini* has been reported to produce hypoglycemic and hypolipidemic effect (Ravi, Rajasekaran et al. 2005) in STZ-diabetic rats. *Syzygium cumini* was produced prominent fall of blood sugar in mice (Villasenor and Lamadrid 2006). Aqueous and ethanolic extracts of the fruit-pulp has been reported to produce antihyperglycemic effect in alloxan diabetic rats, and 24.4% raise in plasma insulin level in mild diabetic and 26.3% in severely diabetic rabbits (Sharma, Nasir et al. 2006).

### 2.3.7 *Swertia chirayita*: Hindi name (Kirayat Chirata)

The herbs grow abundantly in Himalayan regions of India and are used for treatment of various ailments by the tribes (Grover, Yadav et al. 2002). The ethanol extract of plant potentially lowered the blood sugar level in fasted, glucose fed and tolbutamide pretreated animals (Sekar, Mukherjee et al. 1987). Hexane fraction of this plant, lowered blood sugar of albino rats with increased glycogen content of liver and insulin released from pancreatic β-cells (Chandrasekar, Bajpai et al. 1990). Swerchirin isolated from hexane fraction of plant exerted potent hypoglycemic activity in normal and STZ-diabetic albino rats (Saxena, Bajpai et al. 1991). Effective dose (ED$_{50}$) of *Swertia chirayita* has been reported to be 23.1mg/kg/oral, lower maximum 40% blood sugar level of male albino rats of body weight 140-165g (Bajpai, Asthan et al. 1991). Alcoholic extract of this plant exhibited hypoglycemic effect in alloxan induced diabetic rats (Kar, Choudhary et al. 2003).

### 2.3.8 *Trigonella foenum-graecum*: Hindi name (Methi)

Plants are commonly cultivated throughout India. The leaves are used as vegetable and seeds as spice. Major alkaloid trigonellin from fenugreek seeds produced hypoglycemic activity (Shani, Goldsehmied et al. 1974). Ethanol extract of leaves has been observed to reduce blood glucose concentration in alloxan induced diabetic rats. Lethal doses (LD$_{50}$) of aqueous leaf extract were 1.9 g/kg at intra-peritoneal and 10g/kg at oral administration (Barry, Hassan et al. 1997). 4-Hydroxyisoleucine, an insulinotropic compound isolated from seeds increased the insulin release in glucose fed hyperglycemic
rats and humans (Sauvaire, Petie et al. 1998). Seeds powder treatment normalized the enhanced lipid peroxidation and reduced the susceptibility to oxidative stress associated with depletion of antioxidants in liver of rats (Anuradha and Ravikumar 2001). Maximum 46.6% decrease in blood sugar level of diabetic rats was observed at oral administration of seed extract (Vats, Yadav et al. 2003). From fenugreek seeds, the soluble dietary fibre (SDF) fraction at (0.5g/kg, orally administered twice daily, for 28 days) inhibited platelets aggregation in type 2 diabetic rats and produced beneficial effect in dyslipidemia (Hannan, Rokeya et al. 2003). Restored activity of glutamate dehydrogenase, NAD linked isocitrate dehydrogenase and D-b-hydroxybutyrate dehydrogenase reported at oral administration of seed powder (5%, for 3 weeks) in alloxan diabetic rats. It also repaired the liver and kidney damage caused by alloxan (Thakran, Siddiqui et al. 2004). 4-hydroxyisoleucine: an amino acid, isolated from seeds, produced anti-hyperglycemic effect and antidyslipidemic activity (Narender, Puri et al. 2006).

2.3.9 *Ficus bengalensis*: Hindi name (Bargad)

Plants grow throughout India and in the Indian tradition it is considered as a holy tree (Mukherjee, Maiti et al. 2006). Leucocyanidin (3-O-β-D-galactosy cellulobioside) a dimethoxy derivative isolated from bark, lowered blood sugar level and increased serum insulin in normal and moderate diabetic rats and it also inhibits the degradation processes of insulin (Kumar and Augusti 1989). Glucoside of leucopelargonidin isolated from bark has been reported to induce hypoglycemic, hypolipidemic and serum insulin raising effect in moderately diabetic rats (Cherian and Augusti 1993). 3-O-α-L-rhamnoside, a dimethoxy derivative isolated from bark, lowered blood sugar in fasted and glucose induced hyperglycemic rats (Cherian, Kumar et al. 1992), along with enhanced insulin secretion from β-cells (Augusti, Rajisuan et al. 1994). Leucodelphinidine derivative isolated from bark exhibited hypoglycemic activity in normal and alloxan diabetic rats (Geetha, Mathew et al. 1994). 3-O-α-L-rhamnoside isolated from the bark has been showed its median effective dose (ED$_{50}$) as 100mg/kg with 12% hypoglycemic action in normal rats (Cherian and Augusti 1995). Hypoglycemic and hypercholesterolemic effect of aqueous bark extract was observed in alloxan induced mild and severe diabetic rabbits. Leucopelargonin isolated from bark lowered the fasting blood sugar (34%) and glycosylated haemoglobin (28%) of alloxan diabetic dogs (Daniel, Devi et al. 2003).
Table: Classification of plants used as herbal antidiabetics: Possible mode of actions

<table>
<thead>
<tr>
<th>Mechanism of action</th>
<th>Name of the plant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Insulin mimetic</strong></td>
<td><em>Momordica charantia</em></td>
</tr>
<tr>
<td></td>
<td><em>Sambucus nigra</em></td>
</tr>
<tr>
<td><strong>Insulin secretagogues</strong></td>
<td><em>Panax ginseng</em></td>
</tr>
<tr>
<td></td>
<td><em>Pterocarpus marsupium</em></td>
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<tr>
<td></td>
<td><em>Gymnema sylvestre</em></td>
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<tr>
<td></td>
<td><em>Trigonella foenum graecum</em></td>
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<tr>
<td></td>
<td><em>Swertia chirayata</em></td>
</tr>
<tr>
<td></td>
<td><em>Andrographis paniculata</em></td>
</tr>
<tr>
<td></td>
<td><em>Ocimum sanctum</em></td>
</tr>
<tr>
<td><strong>Increasing glucose uptake</strong></td>
<td><em>Swertiya chirayata</em></td>
</tr>
<tr>
<td></td>
<td><em>Ocimum sanctum</em></td>
</tr>
<tr>
<td></td>
<td><em>Swertia japonica</em></td>
</tr>
<tr>
<td><strong>Inhibit aldolase reductase activity</strong></td>
<td><em>Aralia elata</em></td>
</tr>
<tr>
<td></td>
<td><em>Atractylodes laotana</em></td>
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<td></td>
<td><em>Phellodendron amurense</em></td>
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<td></td>
<td><em>Paeonia latifolia</em></td>
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<td></td>
<td><em>Glycyrrhiza glabra</em></td>
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<tr>
<td></td>
<td><em>Comus macrophylla</em></td>
</tr>
<tr>
<td><strong>Increasing glucose utilization/glycogen formation</strong></td>
<td><em>Gymnema sylvestre</em></td>
</tr>
<tr>
<td><strong>Increase of glucose transporter protein (glut4) in muscle</strong></td>
<td><em>Nelumbo nucifera</em></td>
</tr>
<tr>
<td></td>
<td><em>Panax ginseng</em></td>
</tr>
<tr>
<td></td>
<td><em>Swertiya chirayata</em></td>
</tr>
<tr>
<td><strong>Inhibit gluconeogenesis</strong></td>
<td><em>Panax ginseng</em></td>
</tr>
<tr>
<td></td>
<td><em>Dioscorea batatas</em></td>
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<td></td>
<td><em>Cinnamomum cassia</em></td>
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<td></td>
<td><em>Glycerihiiza uralensis</em></td>
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<tr>
<td></td>
<td><em>Bauhinia meglandra</em></td>
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<tr>
<td></td>
<td><em>Trigonella foenum graecum</em></td>
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<td></td>
<td><em>Syzygium cumini</em></td>
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</table>
2.4 Biochemical & molecular targets for the action of antidiabetics

2.4.1 Reducing excessive hepatic glucose production

The regulation of hepatic gluconeogenesis is an important process in the adjustment of the blood glucose level, and pathological changes in the glucose production of the liver are a central characteristic in type 2 diabetes. The principal parameters affecting hepatic glucose output are the concentrations of the available glucogenic substrates and the activity of a few regulatory enzymes. The activity of the key gluconeogenic enzymes phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G-6-Pase) is regulated by transcriptional and nontranscriptional mechanisms, whereas the third key enzyme fructose-1, 6-bisphosphatase (FBPase) is also regulated through competitive inhibition by fructose 2, 6-bisphosphate.

Insulin is the most important hormone that inhibits gluconeogenesis. It acts predominantly by suppressing the expression of the genes for the key gluconeogenic enzymes PEPCK and G-6-Pase. PEPCK catalyzes one of the rate-limiting steps of gluconeogenesis, the reaction of oxaloacetic acid to phosphoenolpyruvate, whereas G-6-Pase catalyzes the final step of gluconeogenesis, the production of free glucose from glucose 6-phosphate. The expression of the genes for G-6-Pase and PEPCK is induced by glucagon during fasting, by glucocorticoids during periods of stress, or by catecholamines during exercise (Barthel and Schmol 2003).

Gluconeogenesis and glycogenolysis play a very important role in regulating endogenous glucose production by synthesis or breakdown of glycogen. Increased rates of hepatic glucose production are largely responsible for development of overt hyperglycaemia. Glucagon contributes to hyperglycaemia through induction of gluconeogenesis and glycogenolytic pathways its receptor; a seven transmembrane domain G-protein receptor could be a target for development of small-molecules antagonists. In addition, several enzymes that regulate rate-controlling steps in gluconeogenesis or glycogenolytic pathways can also be used as molecular target for therapeutic intervention. One such enzyme is inhibition of hepatic glycogen phosphorylase (Treadway, Mendys et al. 2001), an enzyme that catalyses release of glucose from glycogen. Others are fructose-1, 6-bisphosphatase and glucose-6-bisphosphatase (Moller 2001). Whereas inhibition of fructose 1, 6-bisphosphatase would selectively block gluconeogenesis by disrupting the conversion of fructose-1, 6-bisphosphate to fructose-6-phosphate, inhibition of glucose-6-phosphatase would attenuate final step in hepatic glucose production common to gluconeogenic and glycogenolytic pathways.
Fig. 1: In the hepatocyte, insulin stimulates the utilization and storage of glucose as lipid and glycogen, while repressing glucose synthesis and release.

Inhibition of Hepatic Glycogen phosphorylase is a promising treatment strategy for attenuating hyperglycemia in Type 2 diabetes (Baker, Timmons et al. 2005). Great interest has been generated regarding potential benefits of glycogen phosphorylase inhibitors for treatment of T2DM because their mode of action may avoid hyperglycemic episodes (Treadway, Mendys et al. 2001; Somsak, Nagya et al. 2003).

One of the probable mechanisms of antidiabetic action of glycogen phosphorylase inhibitors is the suppression of either gluconeogenesis or glycogenolysis to improve hepatic glucose production (HGP). Liver produces glucose by two pathways, gluconeogenesis and glycogenolysis (breakdown of glycogen by phosphorylase). Most of the inhibitors of GP acts by inhibiting glycogenolysis, that improve glycemic control, based on patients with hepatic glycogen storage diseases, where episodic hypoglycemia is observed (Ogawa, Willoughby et al. 2003).

Under normal conditions cellular glucose is phosphorylated by hexokinase for further utilization through glycolysis or the Hexose monophosphate shunt (HMPS) pathway. During hyperglycemia cellular levels of glucose greatly increase in tissues where glucose entry is independent of insulin. In these tissues, which include lens, retina, kidney, and peripheral nerves, this excess glucose is metabolized via an accessory pathway known as the polyol pathway (Kinoshita 1990).

Aldose reductase is the rate-limiting enzyme of the polyol pathway. AR catalyzes glucose to sorbitol. Sorbitol dehydrogenase, the second enzyme of the pathway, further
converts sorbitol to fructose (Kinoshita 1990). An AR-catalyzed formation of sorbitol was also observed in a number of tissues and the polyol pathway was recognized as an essential component of intermediary metabolism (Bhatnagar, Ruef et al. 2001). Although some recent studies implicate a role for AR in the detoxification of aldehydes, the major bioactive products of lipid peroxidation (Dixit, Puri et al. 2001), the physiological relevance of this pathway and its role in overall carbohydrate metabolism remains unclear.

Under normoglycemic conditions, approximately 3% of the glucose metabolized is routed via the polyol pathway (Morrison, Fletcher et al. 1989). However, under hyperglycemic conditions, this pathway accounts for more than 30% of the glucose utilized (Gonzalez, Sochor et al. 1983). The production of galactitol and sorbitol (polyols of galactose and glucose) in large quantities in rat lens during sugar induced cataractogenesis stimulated intense interest in the pathological role of polyol pathway in the development of cataract. Reduction of excess glucose to the osmolyte sorbitol leads to osmotic swelling, changes in membrane permeability, and subsequent cataract formation. Therefore, prevention of polyol accumulation by inhibiting AR to prevent cataract and other diabetes-associated ocular pathology has received considerable attention (Suryanarayana, Kumar et al. 2004).

2.4.2 Treating \(\beta\)-cells

Two distinct gut derived peptide hormones glucagon-like-peptide-1 (GLP-1) and gastric inhibitory peptide (GIP) act through their respective G protein coupled receptors on \(\beta\)-cells to potentiate glucose stimulated insulin secretion (Drucker 2008). Administration of any of these two hormones to humans can potentiate insulin secretion. Since both hormones are subject to rapid amino terminal degradation by dipeptidylpeptidase-IV (DPP-IV), use of modified GLP-1 peptide agonists resistant to this enzyme has been recommended. It is observed that DPP-IV null mice have increased circulating active GLP-1 along with enhanced insulin secretion, and an otherwise healthy phenotype. Thus, development of GLP-1 analogues and DPP-IV inhibitors is likely to yield important new therapeutic approaches that might circumvent the liabilities of hypoglycaemia, weight gain and secondary failures associated with sulphonylurea use.

2.4.3 Insulin receptor mimetics

Insulin mimetic was thought to replace the need for daily insulin injections with a more palatable option. In 1999, 14 years after the cloning of insulin receptor, Merck Research Laboratories identified a small molecule fungal metabolite from *Pseudomassaria sp.*, L-783281 (Fig 1.) from cell based screening assay. This compound was shown to be a selective insulin receptor activator that mimicked insulin effects including phosphorylation
of insulin receptor substrate-1 (IRS-1), activation of PI3K and Akt, increased glucose uptake in skeletal muscle and adipocytes. Oral administration to db/db and ob/ob mice resulted in significant decrease in blood glucose levels (Zhang, Salituro et al. 1999). Other compounds, which are apparently insulin sensitizer rather than mimetics, will be described in subsequent sections. Proof of concept for small molecule insulin mimetics holds significant promise as an alternative treatment for diabetic patients.

Fig. 1: Chemical structure of L-783281.

2.4.4 Insulin sensitizers
Different new targets as well as new molecules for old targets are being exploited by the pharmaceutical companies in this area. There are many front-runners in this area. The modification of existing therapy i.e., PPAR agonists, is still persuaded in a big way worldwide.

2.4.4.1 PPAR Agonists
Nuclear receptors function as ligand-activated transcription factors that regulate the expression by binding directly to the DNA of their target genes. Each receptor recognizes a specific DNA sequence, called a hormone response element (HRE), which is usually located in the promoter region of the target gene. Nuclear receptors mediate the actions of numerous hormones and polypeptides and affect processes as diverse as development, homeostasis and energy metabolism. The PPARs of nuclear receptors mainly consist of three subtypes (PPARα, PPARγ, and PPARδ/β). All three PPAR isoforms possess similar structural and functional features. Principally, four functional domains have been identified, and are referred to as A/B, C, D, and E/F. The N-terminal A/B domain contains ligand-independent activation function 1 (AF-1). The ligand-independent activation region can confer constitutive activity on the receptor, and is negatively regulated by phosphorylation (Werman, Hollenberg et al. 1997). The DNA binding domain (DBD) or C domain consists of two zinc fingers, and is directly involved with the binding of PPAR to the peroxisome proliferator response element (PPRE) in the promoter regions of target
genes. PPREs are direct repeat (DR)-1 elements consisting of two hexanucleotides with the AGGTCA consensus sequence separated by a single nucleotide spacer. Such a sequence, or a similar one, has been found in numerous PPAR-inducible genes, including acyl-CoA oxidase (ACO) and adipocyte fatty acid-binding protein (aP2) (Graves, Tontonoz et al. 1992). The D site is a hinge region and a docking domain for corepressors. The E/F domain or ligand-binding domain (LBD) is responsible for ligand specificity and the activation of PPAR binding to the PPRE, which increases the expression of the targeted gene. Upon the binding of a specific ligand to LBD of the E/F domain, the conformation of a PPAR is altered and stabilized. The ligand-bound LBD results in the recruitment of transcriptional coactivators, resulting in gene transcription. Although three of the PPAR isoforms possess similar structures, it is clear that these receptors perform distinct functions according to the specific ligands and their expression patterns in the tissues.

2.4.4.2 PPAR-α

PPAR-α is mainly expressed in tissues with elevated mitochondrial and peroxisomal fatty acid β-oxidation rates, such as liver, heart muscle, kidney, skeletal muscle, and brown fat. It regulates the expression of proteins involved in the transport and β-oxidation of free fatty acids (FFAs), predominantly in the liver. Specifically, PPAR-α upregulates fatty acid transport protein (FATP), which facilitates the uptake of long chain fatty acids by the liver. After passage across the plasma membrane, the fatty acids are either esterified and activated by acyl-coA synthetase (ACS) to acyl-coA derivates or bound by fatty acid binding protein (FABP). In this way, escape of free fatty acids from the cell is prevented. Both the ACS and the FABP gene contain a PPRE in their promoters. PPAR-α promotes β-oxidation of the activated acyl-CoA esters by inducing the enzymes medium chain acyl-coenzyme A dehydrogenase (MCAD), acyl-coA oxidase (ACO), and cytochrome P450 fatty acid ω-hydroxylase. These are the key enzymes involved in β-oxidation in mitochondria, peroxisomes and microsomes, respectively (Ahmed, Furlong et al. 2007).

PPAR receptors were cloned with the realization that they were responsible for peroxisome proliferation observed in rodent liver after treatment with fibrates. Several groups implicated fatty acids as natural ligands for PPAR-α. A search for natural PPAR-α ligands in human serum identified palmitic acid, oleic acid, linoleic acid and arachidonic acid as endogenous activators of rat PPAR-α (Chakravarthy, Pan et al. 2005). Recently a compound (NS-220, Fig. 2) has been reported as potent and selective PPAR-α agonist as a
promising drug candidate for overall management of metabolic syndrome in type 2 diabetes (Asaki, Aoki et al. 2008). In KK-Ay mice at 1 mg/kg oral dose it lowered triglycerides, blood glucose and VLDL by 71%, 21% and 50% respectively, while raising HDL by 34%. Eli Lilly has a triazolone based highly potent and selective PPAR-α agonist (LY-518674, Fig. 2) (Millar, Duffy et al. 2009), which is in phase I clinical trial.

![Structures of NS-220 and LY-518674](image)

Fig. 2: Structures of NS-220 and LY-518674

### 2.4.4.3 PPAR-β

PPAR-β is expressed in a range of tissues. The natural ligand of this nuclear receptor remains unclear, although long chain fatty acids have been proposed as candidates (Carvajal, Hernandez-Esquivel Mde et al. 2007). Recent reports have indicated the beneficial effect of PPAR-β modulation in various disease conditions associated with metabolic syndrome. Miyachi and coworkers have suggested that PPAR-β agonists increase reverse cholesterol transport (Miyachi 2007). In another recent report Fan and coworkers (Fan, Wang et al. 2008) indicated that PPAR-β ligands could attenuate inflammation and slow down the progression of atherosclerosis. These two reports demonstrated the potential of PPAR-β agonists for amelioration of cardiovascular disorders. Riserus and coworkers (Riserus, Sprecher et al. 2008) have indicated that PPAR-β serves as a widespread regulator of fat burning and thus could be a target for the treatment of obesity and associated.

### 2.4.4.4 PPAR-γ

PPAR-γ is mainly found in adipose tissues (Medina-Gomez, Gray et al. 2007). At low concentration, it is also expressed in skeletal muscles and endothelium. Prominent among modulators of the PPAR-γ is a class of drugs known as glitazones. The most pronounced effect of PPAR-γ modulation by TZDs is the promotion of adipocyte differentiation resulting in change in body composition towards peripheral rather than the central obesity.

This is the only component of the insulin-signaling cascade that has been shown to be up regulated in response to TZDs, and as such offers a direct effect of these compounds.
on insulin sensitivity. Prostaglandin J2 (Kimura, Li et al. 2008) and polyunsaturated fatty acids (Franks, Jablonski et al. 2007) have been discovered as endogenous ligands for PPAR-γ. The PPAR-γ gene produces 2 proteins, PPARγ-1 and the nearly adipose-specific PPARγ-2. PPARγ-2 has 28 additional N-terminal amino acids that confer a 5- to 6-fold increase in transcription-stimulating activity of the ligand-independent activation function-1 domain (Vidal-Puig, Jimenez-Linan et al. 1996). PPARγ also plays a key role in the entraining of adipose tissue lipid metabolism to nutritional state. Two PPARγ compounds are in the advanced stages of clinical trials—balaglitazone (fig. 3) from Dr. Reddy's/Novo Nordisk and FK-614 from Fujisawa (Larsen, Lykkegaard et al. 2008).

Fig. 3: Chemical structures of PPARγ ligands

2.4.4.5 Dual PPAR-α/γ agonist

In spite of the impact the TZDs have made on the clinical management of type 2 diabetes, they were unable to take care of lipid profiles. In addition to the characteristic combination of insulin resistance and insulin deficiency, the type 2 diabetic often displays cardiovascular risk factors including dyslipidemia, hypertension and obesity. The recent publication of the UKPDS (Cugnet-Anceau and Bauduceau 2009) has revealed that in type 2 diabetes, intensive glucose lowering therapy is ineffective at reducing cardiovascular complications, despite decreasing microvascular complications such as retinopathy. The profile of a dual PPAR-α/γ agonist appears well suited as a treatment for type 2 diabetes (Tanabe, Tamasawa et al. 2008) because of the insulin sensitizing/glucose-controlling potential of PPARγ agonists, the molecular target of TZDs, in combination with the positive lipid and cholesterol modulating activities of PPAR-α agonists, the molecular target of the fibrates (Chang, Jaber et al. 2007). To fulfill this dual action a new class of compounds, alpha-alkoxyphenylpropanoic acid derivatives, were made and tested. This subtle change in 'pharmacophore' rendered the compounds with additional pronounced activities of PPAR-α. It is pertinent to note that troglitazone and pioglitazone have later been found to provide PPAR-α agonist property although at very high concentrations.
The first of its kind is ragaglitazar from Dr. Reddy’s Laboratories/Novo Nordisk, which is an alpha-alkoxyphenylpropanoic acid derivative (fig.4). Although this compound showed an excellent profile in sensitizing insulin, lowering blood glucose and free fatty acid, elevating HDL-cholesterol in phase II clinical trials (Miyachi 2004), due to some incidence of bladder tumor in rodents, the trials have been discontinued. Tesaglitazar, another propanoic acid derivative dual activator is still in phase III clinical trials. A dual PPAR-α/γ agonist from Eli Lilly (LY-510929, Fig.4), has demonstrated comparable to superior efficacy compared to rosiglitazone in a 12 wk phase II study. Another balanced PPAR-α/γ dual activator muraglitazar, a benzylglycine derivative, has entered phase III clinical trials for type 2 diabetes by August 2003. In a 26 days phase II study 24-h mean glucose and fasting glucose reduction were greater in subjects treated with >5 mg of muraglitazar than those treated with 45 mg pioglitazone. Muraglitazar was safe and well tolerated up to 20 mg dose level (Cox 2005).

2.4.5 Adipocyte derived proteins: viable drug targets?

The therapeutic potential of adipose tissue-derived factors was initially recognized upon the discovery of leptin. Leptin is an adipocyte-specific protein that acts as satiety signal from fat to the brain (Friedman 2002). In addition, several hormonally active factors have been discovered that are produced uniquely in adipocytes and might serve directly as drug targets (Gimeno and Klaman 2005). Many of these proteins, referred to as adipokines, play a role in obesity-related metabolic complications. The release of
adipokines from fat is regulated as a function of the system’s metabolic state and in response to inflammatory signals. Therefore, conditions of abnormal adipose tissue mass result in the dysregulation of adipokines (Rajala 2003). Replenishment of recombinant forms of adipokines that are downregulated in obesity or the depletion of selective adipokines that are produced in excess in the obese state might have a powerful therapeutic impact. Alternatively the stimulation or the reduction of the release of these proteins from adipocytes could represent an additional approach to alter circulating levels of these proteins.

**Pro-hyperglycaemic**

- Resistin
- TNF-α, IL-6, other cytokines
- RBP4

**Anti-hyperglycaemic**

- Leptin
- Adiponectin
- Visfatin
- Omentin

*Fig.*: Adipocyte-derived proteins with anti-diabetic action (green arrows) include leptin, adiponectin, omentin and visfatin. Other factors tend to raise blood glucose (red arrows), including resistin, TNF- and RBP4. TNF- and human resistin are probably secreted by non-adipocytes within the fat pad. IL, interleukin.

### 2.4.5.1 Leptin

To date, the success of adipokine therapy has been somewhat limited (Gorden and Gavrilova 2003). Leptin supplementation is currently used clinically to treat patients with congenital leptin deficiency. These patients are hyperphagic and morbidly obese and the administration of recombinant leptin leads to a dramatic reduction of body weight (Licinio 2004). Leptin deficiency is also found in acquired and inherited lipodystrophy that is accompanied by fat loss in selective areas of the body. These rare conditions are associated with severe insulin resistance, dyslipidemia and hepatic steatosis. Leptin-replacement therapy in these patients leads to a correction of the hyperglycemia and a reduction in plasma lipids, thus helping to minimize the risk of metabolic complications. Nevertheless, the results of clinical trials with recombinant leptin as anti-obesity drugs remained disappointing. In generalized obesity, administration of leptin is ineffective.
because leptin levels increase in proportion to adipose mass and are relatively high in obese patients. Leptin resistance rather than lack of circulating leptin is the underlying reason, such that leptin replacement therapy is of limited value in this context (Montez 2005).

2.4.5.2 Adiponectin

A promising therapeutic adipokine is adiponectin. Adiponectin (also known as Acrp30) is a complex protein secreted specifically from adipocytes. Low serum levels of adiponectin are causally linked to insulin resistance and are predictive for the development of diabetes and cardiovascular disease. A sexual dimorphism exists for plasma adiponectin levels with significantly higher concentrations in the serum of females. Obesity is associated with decreased adiponectin levels (Hotta 2000). Promising results have been obtained for adiponectin as a therapeutic agent in numerous animal experiments and human epidemiological studies. Adiponectin is a multifunctional protein with protective roles against the development of insulin resistance, dyslipidemia, nonalcoholic fatty liver disease, atherosclerosis, cardiac hypertrophy and ischemic injury (Shibata 2004). Beyond that, adiponectin has been shown to act in the brain to decrease body weight (Qi 2004). However, the understanding of the molecular mechanisms by which adiponectin achieves these effects is still poor. The production of recombinant adiponectin is challenging because of the complex tertiary and quaternary structure of the protein and the distinct activities of the different isoforms. Adiponectin, like other adipokines (such as resistin), is secreted from the adipocyte as distinct complexes (Patel 2004). Adiponectin can form trimers (the basic building block for the higher-order complexes), hexamers consisting of two trimers, and higher molecular-weight forms (HMW) consisting of up to 12–18 subunits (Pajvani 2003). Proper folding and assembly into these higher-order structures depend on posttranslational modifications that can be achieved only in mammalian production systems. Adiponectin contains a collagenous tail domain and a globular head domain. The subunits are linked through intermolecular cysteine bonds. A globular form of adiponectin produced in Escherichia coli possesses biological activity distinct from the mammalian produced full-length protein. Whereas a pharmacological dose of the latter improves hepatic insulin sensitivity in lean mice and even more effectively in obese animals (Berg 2001). The former was shown to activate fat oxidation in skeletal muscle (Yamauchi 2003). Interestingly, a mutant version of adiponectin that lacks a critical cysteine residue in the collagen domain is considerably more bioactive than wild-type adiponectin (Pajvani 2003). Mice overexpressing this mutant form of adiponectin
(Cys39Ser) in a genetically obese background (leptin deficient ob/ob mice) display massive adipose tissue accumulation (unpublished observations from our laboratory). These morbidly obese animals are however metabolically very healthy with nearly normal glucose and lipid levels. These observations suggest a processing step in the activation cascade for adiponectin that converts the HMW form into the smaller short-lived trimer, possibly involving a serum reductase or protease. Clearly, many fundamental questions remain to be answered on the relationship between structure and function of adiponectin (Pajvani 2003).

2.4.5.3 Resistin and resistin-like molecules

Resistin has been proposed as another adipokine involved in the complex etiology of insulin resistance (Rajala 2003). In rodents the cysteine-rich 10 kDa protein resistin is secreted mainly from adipocytes, whereas in humans its main source is peripheral-blood mononuclear cells (Patel 2003). In contrast to adiponectin, resistin levels increase approximately two fold in mice fed a high-fat diet, leading to severe hepatic insulin resistance (Muse 2004). The neutralization of resistin with a specific antibody or the downregulation of resistin to normal levels via antisense technology is effective in re-establishing normal insulin sensitivity in these rodent models (Muse 2004). Although the unusual hexameric tail-to-tail conformation of resistin and its closest family member resistin-like molecule (RELM) β suggests a receptor clustering mechanism of activation, the discovery of corresponding receptors remains elusive (Patel 2004). Intriguingly, infusion of the intestinally produced RELM β has similar effects on hepatic insulin sensitivity as does the infusion of the adipocyte-derived resistin, suggesting related signaling mechanisms between nutrient absorbing tissues and nutrient-storing tissues (Rajala 2003). Although the functional role of resistin in humans is not well established, this small hormone represents an interesting candidate from a pharmacological perspective. As it is the case for adiponectin, minor variations in the circulating levels of these adipokines might have potent therapeutic effects.

2.4.5.4 Visfatin

The connection between visceral adiposity and insulin resistance has led several groups to try to identify secreted products derived specifically from this depot. The first such protein reported was visfatin, which had been identified in immune cells years earlier as pre-B-cell colony-enhancing factor (PBEF) (Fukuhara 2005). There were several surprising aspects surrounding this discovery, including the fact that visfatin does not promote insulin resistance-on the contrary, it has a salutary effect on glucose uptake.
mediated by direct binding and activation of the insulin receptor. Visfatin circulates at concentrations well below those of insulin (<10%), however, and fasting and feeding do not regulate its expression, making it doubtful that visfatin alone is an important factor in insulin-receptor signalling. Other aspects of visfatin biology require further study. For example, serum levels of visfatin are variably correlated with type 2 diabetes and other insulin-resistant states (Stephens and Vidal-Puig 2006). Visfatin also has no signal sequence and has been shown to have enzymatic activity as a nicotinamide phosphoribosyltransferase with residence in the nucleus and cytosol (Yang and Chuang 2006). It is not clear whether there is regulated secretion of visfatin or whether serum levels reflect leakage from dead or damaged cells.

2.4.5.5 Omentin

Omentin is another peptide secreted predominantly by visceral fat. Like visfatin, it has positive effects on glucose uptake, although omentin works as an insulin sensitizer and does not have insulin-mimetic properties (Yang and Chuang 2006). Unlike visfatin, omentin seems to be made by stromal-vascular cells within the fat pad rather than adipocytes. Interestingly, omentin is produced in considerable quantities by adipose tissue in humans and macaques but not mice (Yang and Chuang 2006). Omentin's mechanism of action, including target tissues, a receptor or relevant signal transduction pathways, remains obscure.

2.4.5.6 Tumour necrosis factor-α and other cytokines

Tumour necrosis factor-α (TNF-α) was the first secreted adipose protein to be shown to have effects on glucose homeostasis (Hotamisligil 1999). TNF-α levels are elevated in obesity and in other insulin-resistant states (such as sepsis), addition of TNF-α to cells and mice reduces insulin action, and blockade of TNF-α action by biochemical or genetic means restores insulin sensitivity in vivo and in vitro. Interestingly, TNF-α seems to be derived from cell types other than adipocytes themselves. Macrophages in particular have been implicated in TNF-α production from murine fat pads (Xu 2003). Other cytokines, including interleukin-6, are produced by adipocytes, and there is conflicting evidence suggesting that they have both insulin-resistance-promoting and insulin-sensitizing effects (Rotter, Nagaev et al. 2003). Such 'adipocytokines' can promote insulin resistance through several mechanisms. These include c-Jun N-terminal kinase 1 (JNK1)-mediated serine phosphorylation of insulin receptor substrate-1 (IRS-1), IκB kinase (IKK)-mediated nuclear factor-κB (NF-κB) activation, induction of suppressor of cytokine signalling 3 (SOCS3) and production of ROS (Howard and Flier 2006).
2.4.6 Protein Tyrosine Phosphatase Inhibitors

The interaction of insulin with its receptor leads to phosphorylation of certain tyrosine moieties (Tyr 1146, 1150 and 1151) within the receptor protein, thus activating the receptor kinase. Protein tyrosine phosphatases (PTPases) dephosphorylate the activated IR, attenuating the tyrosine kinase activity. Thus, PTP1B specific inhibitors are expected to enhance insulin sensitivity and act as effective therapeutics for the treatment of type 2 diabetes, insulin resistance and obesity. Although there are a number of molecules in discovery and early preclinical stages, there is no single compound in development. Ertiprotafib (fig. 5) was being developed by Wyeth (formerly Wyeth-Ayerst); however, development was discontinued after phase II in June 2002. One benzoic acid derivative (6) from Abbott and a series of peptidomimetics, of which 7 (fig. 5) is a lead from Biovitrum, are being investigated for treatment of diabetes. The issue of specificity in developing protein phosphatase inhibitors has been raised as residues in protein kinases, which interact with ATP, are conserved. A principal challenge that lies beyond the discovery and characterization of novel PTPases will be the ability to select the appropriate target enzyme for modulation.

Fig. 5: Chemical structures of some PTP1B inhibitors.

2.4.7 Inhibition of 11-β hydroxysteroid dehydrogenase type 1

11-β hydroxysteroid dehydrogenase enzymes (11β-HSD) type 1 and 2 regulate the activity of glucocorticoids and mineralocorticoids at the pre-receptor level (Tomlinson 2004). The type 1 isoform (11β-HSD1) has received increasing attention recently thanks to its putative role in the development of the metabolic syndrome. 11β-HSD1 expression is
rather ubiquitous throughout the body, with highest levels in the liver, adipose tissue and the brain, and protein levels are subject to regulation by inflammatory mediators such as TNF-α and interleukin (IL) 1β (Kostadinova 2005). The enzyme functions primarily as a reductase converting hormonally inactive cortisone into the high-affinity ligand cortisol. Glucocorticoids are potent inducers of adipocyte differentiation in in-vitro systems and 11β-HSD1 activity increases during adipogenesis. Accordingly, an excess of glucocorticoids is associated with the development of obesity. This becomes apparent in patients with the Cushing’s syndrome, a condition of pathophysiologically elevated glucocorticoids resembling the metabolic syndrome (Seckl 2004). Transgenic mice overexpressing 11β-HSD1 selectively in adipose tissue also display the features of the metabolic syndrome (Masuzaki, Paterson et al. 2001). Interestingly, 11β-HSD1 knockout mice are resistant to high-fat diet induced obesity and have reduced resistin and elevated adiponectin levels, suggesting a possible indirect insulin sensitizing effect of a pharmacological inhibition of this enzyme (Morton 2004).

11beta-Hydroxysteroid dehydrogenases (11beta-HSDs) are key enzymes regulating the pre-receptor metabolism of glucocorticoid hormones, which play essential roles in various vital physiological processes. The modulation of 11beta-HSD type 1 activity with selective inhibitors has beneficial effects on various conditions including insulin resistance, dyslipidemia and obesity. Therefore, inhibition of tissue-specific glucocorticoid action by regulating 11beta-HSD1 constitutes a promising treatment for metabolic and cardiovascular diseases (Su, Vicker et al. 2009). Novel antidiabetic arylsulfonamidothiazoles were presented by Barf and co-workers, which exert action through selective inhibition of the 11β-HSD1 enzymes, thereby attenuating hepatic gluconeogenesis.
2.4.8 Insulin secretagogues

2.4.8.1 Glucagon-Like Peptide-1 (GLP-1)

Glucagon-like peptide-1 (GLP-1) is an incretin peptide hormone released upon absorption of nutrients from the endocrine (L) cells of the distal intestinal mucosa followed by glucose-dependent increase in secretion of insulin. This 30-amino acid peptide is known to improve insulin synthesis and secretion, stimulate β-cell growth, promote satiety, delay gastric motility, increase glucose disposal in fat, muscle and liver, improve insulin receptor binding and decrease glucagon and gluconeogenesis, all of which collectively contribute to overall normalization of glucose level (Efendic, Alvarsson et al. 2008). All of these effects taken together put GLP-1 to be an obvious drug candidate for the treatment of type 2 diabetes. Apart from food intake lowering the glucagonostatic effect is particularly interesting as type 2 diabetic patients are characterized by increased plasma glucagon levels, which again leads to an increased hepatic glucose output (Muscelli, Mari et al. 2008). The stimulatory effect of the cleavage fragment (7-36 amide) of proglucagon produced by small intestinal L-cells on insulin release is the result of multiple effects on the stimulus-secretion coupling chain.

Near normalization of diurnal plasma glucose concentrations were obtained during continuous intravenous infusion of GLP-1 in type 2 diabetic patients (Ahren 2007). GLP-1 is metabolized extremely rapidly (half-life is ~ 5 min) by the ubiquitous enzyme Dipeptidyl peptidase IV (DPP-IV), which cleaves a dipeptide from GLP-1 and thereby inactivates it. In fact, the metabolite of GLP-1 may act as a GLP-1 receptor inhibitor (Salvatore, Carbonara et al. 2007). As this peptide must be administered parenterally, this may limit its use. Again very short half-life restricts its use as drug. Slow release formulations and sublingually absorbed form of GLP-1 are being considered as effective agent for the treatment of type 2 diabetes.

2.4.8.2 Dipeptidyl Peptidase-IV Inhibitors

Inhibition of DPP-IV, a ubiquitous yet highly praline specific serine protease that cleaves N-terminal dipeptides from polypeptides with L-proline or L-alanine at the penultimate position, is a novel therapeutic approach to the treatment of type 2 diabetes (Yoshida, Sakashita et al. 2007). It is also known as lymphocyte cell surface protein CD26, which is a widely expressed glycoprotein that exhibits three principal biological activities: in humans it acts as an adenosine deaminase (ADA)-binding protein; it contributes to extra cellular matrix binding and it exhibits post proline or alanine peptidase activity. The active form of the incretin hormone GLP-1 (GLP-1[7-36] amide) falls rapidly
following postprandial excursion to its inactive form (GLP-1[9-36] amide) with a half-life of approximately one minute. DPPIV is thought to be the primary enzyme responsible for this hydrolysis (Sebokova, Christ et al. 2007) via cleavage at the N-terminal region after L-proline or L-alanine, although, to a lesser extent, neutral endopeptidase is also responsible. Inhibition of DPP-IV, therefore, is expected to significantly reduce inactivation of GLP-1 and should lead to an increase in circulating levels of the active form of the hormone. Supporting evidence for this comes from DPP-IV deficient mice, which have elevated levels of GLP-1. DPP-IV inhibitors act as indirect stimulators of insulin secretion by stabilization of GLP-1.

2.4.9 AMPK Activators

Metabolic syndrome is characterized by a cluster of metabolic disorders, such as reduced glucose tolerance, hyperinsulinemia, hypertension, visceral obesity and lipid disorders. The benefit of exercise in maintaining total metabolic control is well known and recent research indicates that AMP-activated protein kinase (AMPK) may play an important role in exercise-related effects. AMPK is considered as a master switch in regulating glucose and lipid metabolism. AMPK is an enzyme that works as a fuel gauge, being activated in conditions of high phosphate depletion. In the liver, activation of AMPK results in decreased production of plasma glucose, cholesterol, triglyceride and enhanced fatty acid oxidation. AMPK is also robustly activated by skeletal muscle contraction and myocardial ischemia, and is involved in the stimulation of glucose transport and fatty acid oxidation by these stimuli. In adipose tissue, activated AMPK inhibits deposition of fat, but enhances breakdown and burning of stored fat, resulting in reduction of body weight. The two leading diabetic drugs, namely metformin and rosiglitazone, and adipokines, such as adiponectin and leptin, show their metabolic effects partially through AMPK. These data suggest that AMPK may be a key player in the development of new treatments for obesity, Type 2 diabetes and the metabolic syndrome. In this review, the author provide insight into the role of AMPK as a probable target for treatment of metabolic syndrome (Misra 2008).

Recent data suggest that the effects of metformin, adiponectin, and other agents that regulate glucose and free fatty acid metabolism may be mediated at least in part via AMPK activation (Misra 2008). Chronic activation of AMPK mimics several effects of exercise in skeletal muscle, such as induction of mitochondrial oxidative enzymes, enhancement of glucose transport, and induction of GLUT4 expression (Karlsson, Chibalin et al. 2009). Given that some of the targets of AMPK phosphorylation are
involved in the control of lipid and lipoprotein metabolism, AMPK activation in diabetics might favorably modulate lipid metabolism as well as glucose metabolism.

2.5 Possible New Therapy for Diabetes

2.5.1 Tyrosine kinase inhibitors reverse type 1 diabetes

Type 1 diabetes (T1D) is an autoimmune disease dependent on T-cell-mediated destruction of insulin-producing β-cells. Disease progression is strongly dependent on T-cells, B-cells and macrophages. Importantly, recent studies have emphasized a role of inflammatory processes in β-cell destruction and insulin resistance. To date there is no good immunotherapy to treat or prevent the development of this disease. T1D is characterized by the development of autoreactive antibodies and destructive T-cell infiltration of insulin-producing islet β-cells. The NOD (nonobese diabetes) mouse is an important model of autoimmune diabetes. Disease occurs spontaneously and shares many phenotypic and genetic similarities with T1D in human subjects (Anderson and Bluestone 2005). Lymphocyte infiltration of the islets of Langerhans begins at 2-4 weeks of age, progressing from periinsulitis to severe insulitis by 10 weeks of age. Diabetes onset typically occurs at 12-14 weeks in most female NOD mice. Given the overlap between the multiple targets of imatinib, previous results in other models of autoimmunity and the pathogenesis of autoimmune diabetes, we set out to test the hypothesis that this drug might be effective in preventing or treating this autoimmune disease. We show here that imatinib treatment can prevent and even reverse diabetes when administered to NOD mice. Furthermore, imatinib can be administered for as short as 10 weeks with long-lasting effects working through the inhibition of PDGFR. These results, coupled with recent studies demonstrating a direct protective effect of imatinib on type 2 diabetes in rodents (Hagerkvist, Sandler et al. 2007) suggests that this molecule and other kinase inhibitors such as sunitinib have potential as a therapeutic to treat patients with this disease.

2.5.2 Inhibitors of the novel target sodium-dependent glucose co-transporter (SGLT-2) for therapy of type 2 diabetes

The sodium-glucose co-transporter-2 (SGLT2) is a low-affinity transport system that is specifically expressed in the kidney and plays an important role in renal glucose reabsorption in the proximal tubule. Competitive inhibition of SGLT2 therefore represents an innovative therapeutic strategy for the treatment of hyperglycaemia and/or obesity in patients with type 1 or type 2 diabetes by enhancing glucose and energy loss through the urine. The observation that individuals with familial renal glycosuria maintain normal
long-term kidney function provides some reassurance that this mode of action will not adversely affect renal function. Intense research in this therapeutic area has led to the discovery of novel SGLT2 inhibitors, each with different chemical, pharmacodynamic and pharmacokinetic profiles (Idris and Richard 2009).

In the kidney, glucose is freely filtered at the glomerulus and is reabsorbed via active transport mechanisms in the proximal convoluted tubule. Two sodium-glucose co-transporters are responsible for glucose reabsorption: SGLT1 and SGLT2. SGLT1, which is also found in the gut and other tissues, accounts for about 10% of reabsorption. SGLT2 expressed exclusively in the S1 segment of the proximal tubule, accounts for about 90% of reabsorption (Fig. 1). The concentration gradient that drives the action of these transporters is driven by the Na+/ATPase pump and by transport back into the blood via the GLUT2 glucose transporter (Fig. 2). This suggests that the most promising target for drug development is the SGLT2 transporter, both because it is responsible for most glucose reabsorption and because of its exclusive localization to the kidney.

![Diagram of glucose reabsorption in the S1, S2, and S3 segments of proximal tubules.](image URL)

Fig. 1: Sites of glucose reabsorption in the S1, S2, and S3 segments of proximal tubules.
Fig. 2: Mode of action of SGLT1 and SGLT2.

Treating hyperglycemia with drugs that block renal glucose reabsorption via the SGLT2 transporter represents a novel approach to diabetes treatment. Although this approach does not directly target the pathophysiology of type 2 diabetes, it does have an advantage in that the lowering of circulating glucose as a result of urinary glucose loss will result in a net energy deficit, promoting weight loss and potentially indirectly improving many features of the condition, including increased hepatic glucose output and pancreatic β-cell failure. These agents therefore have the potential to be useful add-on agents in patients taking oral hypoglycemic drugs or insulin, with a low risk for hypoglycemia and the potential for weight loss. The results of longer-term clinical trials of safety and efficacy will ultimately determine whether SGLT2 inhibition can be added to the list of drugs that have a place in the management of people with type 2 diabetes.

2.5.3 Adipocyte fatty-acid-binding protein inhibitors: Therapy of type 2 diabetes

Adipocyte fatty-acid-binding protein, aP2 (FABP4) is expressed in adipocytes and macrophages, and integrates inflammatory and metabolic responses. Studies in aP2-deficient mice have shown that this lipid chaperone has a significant role in several aspects of metabolic syndrome, including type 2 diabetes and atherosclerosis.

The adipocyte FABP, aP2 (FABP4), is highly expressed in adipocytes and regulated by PPAR γ agonists, insulin and fatty acids (Hertzel and Bernlohr 2000). Studies in aP2-deficient mice have shown that aP2 has a significant role in many aspects of metabolic syndrome. Deficiency of aP2 partially protects mice against the development of insulin resistance associated with genetic or diet-induced obesity (Uysal, Scheja et al. 2000). Adipocytes of aP2 −/− mice have reduced efficiency of lipid transport in vitro and in vivo, and yet exhibit only minor changes in serum lipids. Interestingly, recent studies demonstrated that aP2 is also expressed in macrophages and regulated by phorbol 12-
myristate 13-acetate, lipopolysaccharide, oxidized low-density lipoproteins and PPAR-γ ligands (Pelton, Zhou et al. 1999; Makowski 2001). The macrophage is a critical site of FABP action, and total or macrophage-specific aP2-deficiency leads to a marked protection against early and advanced atherosclerosis in apolipoprotein E-deficient (Apoe⁻/⁻) mice (Makowski 2001; Boord 2002). These findings indicate an important role for aP2 in the development of major components of metabolic syndrome through its distinct actions in adipocytes and macrophages of integrating metabolic and inflammatory responses. Hence, pharmacological agents that modify FABP function may offer therapeutic opportunities for many components of metabolic syndrome, such as insulin resistance, type 2 diabetes, and atherosclerosis (Masato and Gökhan 2008).

2.5.4 DPP-4 inhibitors for diabetes

Two dipeptidyl peptidase-4 (DPP-4, EC 3.4.14.5) inhibitors are currently used for the treatment of type 2 diabetes: vildagliptin (Galvus®, Novartis International AG, Basel, Switzerland) and sitagliptin (Januvia®, Merck & Co., Inc., Whitehouse Station, NJ, USA). Sitagliptin was approved by the FDA and EMEA for the treatment of T2DM patients who fail to achieve hyperglycemic control with diet and exercise, alone or in combination with another drug such as metformin or a glitazone. At the moment vildagliptin is only approved by the EMEA in Europe for combined treatment with other antidiabetic medications including metformin, sulfonyleureas and thiazolidinediones. Several other DPP-4 inhibitors are expected to become available in the near future; however, given that vildagliptin and sitagliptin were first to be developed, most reports of clinical trials relate to these two compounds.

Sitagliptin and vildagliptin, taken orally once or twice a day at indicated doses, lower postprandial and fasting glucose levels. After prolonged use, T2DM patients have lower glycated hemoglobin levels indicative of an overall reduction of blood glucose over time. Clinical studies also show a significant increase in homeostasis model assessment beta-cell
function index (HOMA-β) and fasting proinsulin/insulin ratio, which are markers of insulin secretion and beta cell function (Garber and Sharma 2008). Whether these results provide evidence for a pancreas-sparing effect of DPP-4 inhibitors in the clinic remains an open question. In general, vildagliptin and sitagliptin are well tolerated. The incidence of hypoglycemia is low and few adverse effects were reported.

The glucose-lowering action of DPP-4 inhibitors derives from the prolongation of the active half-life of the incretins, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). The incretins are peptide hormones secreted by intestinal endocrine cells upon food ingestion. Their immediate effect is to reduce the production of glucagon and to increase insulin secretion by pancreatic beta-cells. DPP-4 proteolytically removes two amino acids from the N-terminal end of the incretins, thereby abolishing the interaction with their cognate receptors. Because DPP-4 is present at the site of production, cleavage of the incretins starts almost immediately after their secretion, and this process has been shown to be an important determinant of the biological half-life of GLP-1 and GIP. Inhibition of DPP-4 increases the life-time of the active forms by a few minutes before they are degraded by other proteases and cleared from the circulation (Deacon, Carr et al. 2008).

A new generation of DPP-4 inhibitors: Research teams in pharmaceutical companies are already developing second generation DPP-4 inhibitors. Extrapolating from vildagliptin and sitagliptin, these molecules will be very potent inhibitors with high selectivity for DPP-4 over other perceived targets. They will sustain high levels of DPP-4 inhibition for prolonged time periods at a relatively low dose taken once a day. Feed-back from post-marketing surveillance will incite discussions between medical practitioners on the type of patient eligible to receive DPP-4 inhibitors as monotherapy or in specific combinations. Some investigators advocate that short-term inhibition of DPP-4 at meal times may benefit specific patient groups and that DPP-4 inhibitors have a potential role in the prevention of T2DM (Lambeir, Scharpe et al. 2008).

2.5.5 Amylin analogs-a novel approach in the treatment of diabetes

Amylin is a peptide hormone that is cosecreted with insulin from the pancreatic β-cell and is thus deficient in diabetic people. It inhibits glucagon secretion, delays gastric emptying, and acts as a satiety agent. Amylin replacement could therefore possibly improve glycemic control in some people with diabetes. However, human amylin exhibits physicochemical properties predisposing the peptide hormone to aggregate and form amyloid fibers, which may play a part in β-cell destruction in type 2 diabetes. This
obviously makes it unsuitable for pharmacological use. A stable analog, pramlintide, which has actions and pharmacokinetic and pharmacodynamic properties similar to the native peptide, has been developed. The efficacy and safety of pramlintide administration has been tested in a vast number of clinical trials.

Pramlintide, a synthetic amylin analog that can be used as a potential adjunctive therapy in patients with type 1 and type 2 diabetes. The rationale for amylin replacement has been defined during the past few years. Several large-scale phase III studies involving more than 3,000 diabetic individuals have demonstrated a beneficial effect of amylin replacement on the HbA1c level in both type 1 and 2 diabetes without an increased number of hypoglycemic events and weight gain. In fact, a significant and sustained weight reduction for 1 year has been observed in type 2 diabetic patients. Pramlintide has been shown primarily to reduce prandial glucose excursions, which have been suggested to play a role in the development of cardiovascular complications, although this still needs to be proven. However, it would be important to further characterize responders to amylin analogs versus nonresponders, in terms of both reduction in HbA1c and weight loss, in order to delineate the group of patients who will profit from treatment. Whether amylin agonists given at bedtime to type 2 diabetic patients could induce “β-cell rest,” analogous to somatostatin and potassium channel openers, also remains to be determined. Pramlintide is currently under evaluation for approval as an adjunctive therapy for insulin-treated diabetic patients (Schmitz, Brock et al. 2004).
SCOPE & PLAN OF PRESENT WORK

The World Health Organization (WHO) has projected that the global prevalence of type 2 DM will more than double from 135 million in 1995 to 300 million by the year 2025. The greatest increase will be in India from 19.4 million to 57.2 million, while in China from 16 million to 37.6 million and USA from 13.9 million to 21.9 million during the same period, unless effective preventive measures are implemented to control this enormous increase. Currently India has got the largest number of diabetic patients and is being called as diabetic capital of the world. As a result, human race will be under serious threat of the disease ‘Diabetes’ in this century. The conventional therapies have limited efficacy, limited tolerability and mechanism-based toxicity. Therefore, the development of new antidiabetic agents with no or minimum side effects is an unmet need.

The work embodied in the present thesis title “Biochemical and molecular targets of novel antidiabetic agents” has been carried out to develop new antidiabetic agents from natural source and plant based synthetic derivatives. For fulfilling this objective, the preclinical studies were carried out in different experimental model of diabetes like Streptozotocin (STZ)-induced β-cell damaged diabetic rats (Type I diabetes model) and C57BL/KsJ-db/db mice (Type 2 diabetic model). In vitro study was also performed for identification of PPARs and LXRs agonist from selected antidiabetic lead molecules using COS-7 mammalian cell line.

Chapter I details the development and validation of streptozotocin (STZ)-induced db/+ mice as an alternate model in antidiabetic drug discovery research.

Chapter II details the antidiabetic effect of natural products isolated from different plants parts in different experimental model of diabetes like streptozotocin (STZ)-induced diabetic rats and type 2 diabetic db/db mice.

Chapter III deals with antidiabetic effect of plant based synthetic isoflavone derivatives in STZ-induced diabetic rats and db/db mice.

Chapter IV deals with the studies about the Mechanism(s) of action of novel lead antidiabetic molecules from natural as well as synthetic origin in db/db mice after multiple oral administration.

Chapter V deals with the studies about the PPARs and LXRs agonist activity and impact on adipocyte differentiation, of selected antidiabetic candidate molecules in COS-7 & 3T3 L1 mammalian cell lines.