INTRODUCTION
A.1 Diabetes - Indian scenario

Diabetes, with its acute and long term complications and associated disorders, is a major health hazard. In keeping with the scenario of most developing countries, India has long passed the stage of a diabetes epidemic. The problem has now reached "pandemic" proportions. An estimated 108 million people in India suffer from endocrine and metabolic disorders and diabetes mellitus accounts for 25 million of this figure. Several of these diseases are caused by environmental factors, are preventable and can also be effectively treated at affordable cost. Yet a majority of them remain undiagnosed and untreated due to the lack of technology use. Unlike in developed countries, endocrine and metabolic disorders are predominantly caused by environmental factors in India and perhaps in other developing countries. Hence their prevalence is several-fold higher in developing countries like India [1].

Diabetes mellitus was known to Indians for several thousand years by the name Madhumeha (from Sanskrit ‘madhu’ meaning sweet/sweetness and ‘meha’ excessive urination) in Ayurveda (ayur life and veda knowledge), the traditional medicine which originated and is practiced in India. Charaka Samhita, the earliest major medical text of ayurveda written by physician Charaka, describes the aetiology, symptomatology, pathology, prognosis and management of disease in detail, where in Madhumeha is classified in the group of urination disorders known as prameha (derived from the root mihsechane in Sanskrit meaning watering or dilution of everything in the body). The aetiology, pathogenesis and the principles of management, which are described in Ayurvedic classics, resemble with the modern concepts. Charaka classified Madhumeha patients into two groups, "Krisha" (Lean Diabetic) and "Sthool" (Obese Diabetic) on very similar grounds as diabetics are classified in IDDM and NIDDM respectively. On a similar pattern we find the classification as Sahja prameha (Congenital) and Apathya nimitaj prameha (due to overeating and wrong eating habits). Following a different approach to begin with, both the groups are treated with specific herbal therapy and diet. Indian Pharmacopoeia describes numerous herbal treatments for diabetes and approximately 85% of the antidiabetic plants widely used around the world are prescribed in India. [2,3]
Global prevalence of diabetes with estimates for the year 2000 and projections for 2030 are described in figures A.1, A.2, A.3, A.4 and Table A.1 [4]. The prevalence of diabetes for all age groups was estimated to rise from 2.8% in 2000 to 4.4% in 2030 and the number of people of with diabetes from 171 million to 366 million. The greatest increase will be seen in India (151%, from 31.7 million to 79.4 million). For developing countries as a whole, the urban/rural ratio in diabetes frequency is predicted to double between 2000 and 2030. Overall, diabetes prevalence is higher in men, but there are more women with diabetes than men. For the developed countries, the oldest age-group (> 65 years)) has the largest number of people with diabetes in 2000 and will experience the greatest increase in numbers by the year 2030. However, for the developing countries, the 45- to 64-year-old age-group contained the largest number of people with diabetes in 2000, and this tendency will be further accentuated by the year 2030. The countries with the largest number of people with diabetes are, and will be in the year 2025, India, China and the U.S. (Table A.1) [4].

Figure A.1. Percentage increase in number of people with diabetes from the year 2000 to 2030
Figure A.2. Percentage increase in total population with diabetes from the year 2000 to 2030

Figure A.3. Percentage change in urban population with diabetes from the year 2000 to 2030
Indians eat less, weigh less and work more than Europeans. But why are they more prone for diabetes than Europeans? In a WHO sponsored study in 1992, Dr. P.V. Rao [5] adopted the 'Thrifty Genotype', a hypothesis based on genetic inheritance put forward by James Neel, a geneticist in 1956. The researchers adopted this to the Indian context and tested among Indians living within India and abroad. Indians have
lived through several centuries of famine and starvation, and largely survived on sustenance foods. Over generations, there evolved a 'thrifty genotype', which made them resistant to prolonged periods of starvation. We tend to store a part of our energy intake simultaneously while 'burning' it. Apparently that may be the reason for a 'big belly' on a small body frame among Indians. The findings of this multinational study by Dr. P.V. Rao were that body fat around waist is the culprit to diabetes and heart disease among Indians. Overall body weight was not always high among Indians with 'big bellies'. This meant that total amount of food intake in an Indian was not high though the contents have changed over centuries from vegetable sources to 'fat rich' animal sources. Even the vegetable oils used for cooking such as coconut oil which is widely used in Kerala, Malaysia and Guyana are strongly related with the high rise in diabetes rates among the populations screened from these areas.

According to a national survey conducted on diabetes, The Prevalence of Diabetes in India Study (PODIS), conducted by ‘Diabetes India’, an association of diabetologists across India, in 96 centres with 41,270 people, the lack of awareness level in Indians who have diabetes is as high as 63.64% (Figure A.5) [6]. The prevalence of diabetes is highest in 40-49 age groups. In the developing countries, diabetes is more in the urban population, because of sedentary life-style and lack of exercise in the urban populace. In the developed countries, diabetes is more in the rural populace as the urban people take precaution of not acquiring diabetes through diet and exercise [7]. In spite of lack of awareness of diabetes and its complications, neither patients nor the general doctors recognize it as a serious health problem. Currently, only 10% of patients in India are getting treatment. Variability of healthcare available in different areas, reluctance of the general public to attend government clinics, lack of education and awareness could be the reasons [7].

There is need for a combined, intensive effort from all concerned to educate people that we (Indians) are more prone to diabetes and cardiovascular complications. Also, to understand that following the dietary restrictions existing in the west blindly would not suit to Indian scenario. It is not how much a diabetic eats or the amount of sugar one consumes, but the nature and quantity of fat present in the food is all that matters. This necessitates an urgent reconsideration of the long-established perception of diabetic diets – “no rice, no fruits, no potatoes, no sugar in coffee/tea, etc.” just does
not mean anything. Instead, “no oils, no fats, no food fads” must be the first dietary advice for a diabetic in India.

![Percentage of diabetic population ignorant of their status in India and Percentage regional distribution of diabetes in India](image)

### Prevalence of Diabetes Mellitus (DM) and Impaired Fasting Glucose (IFG) in rural/urban population (in percentage)

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>All India</th>
<th>Males</th>
<th>Females</th>
<th>Urban</th>
<th>Urban male</th>
<th>Urban female</th>
<th>Rural</th>
<th>Rural male</th>
<th>Rural female</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>3.37</td>
<td>3.35</td>
<td>3.41</td>
<td>4.75</td>
<td>4.74</td>
<td>4.76</td>
<td>1.87</td>
<td>1.81</td>
<td>1.92</td>
</tr>
<tr>
<td>IFG</td>
<td>3.68</td>
<td>3.74</td>
<td>3.63</td>
<td>4.89</td>
<td>4.92</td>
<td>4.83</td>
<td>2.37</td>
<td>2.33</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Figure A.5. The prevalence of diabetes in India study (PODIS 2002)

### A.2 History and overview of diabetes

#### A.2.1 Chronology of some important events in the history of diabetes

- **11th century** – diagnosis of diabetes commonly made by ‘water tasters’, who drank the urine of those suspected of having diabetes. Urine taste of diabetics was thought to be sweet, and the Latin word ‘mellitus’ (meaning ‘honey) was added to the term diabetes.

- **19th century** –
  - development of chemical tests to indicate and measure the presence of sugar in the urine
  - Claude Bernard, a French researcher, studied the functioning of the pancreas and the glycogen metabolism of the liver
⇒ Czech researcher, I.V. Pavlov, discovered the links between the nervous system and gastric secretion, making an important contribution to science's knowledge of the physiology of the digestive system
⇒ 1869 - Paul Langerhans, a German medical student, announced in a dissertation that the pancreas contains two systems of cells. One set secretes the normal pancreatic juice, the function of the other was unknown. Several years later, these cells are identified as the 'islets of Langerhans.'
⇒ 1889 - Oskar Minkowski and Joseph von Mering at the University of Strasbourg, France, first remove the pancreas from a dog to determine the effect of an absent pancreas on digestion
❖ 1908 - German scientist, Georg Zuelzer develops the first injectable pancreatic extract to suppress glycosuria; however, there are extreme side effects to the treatment
❖ 1921 - Isolation of insulin from pancreas in 1921 by Banting and Best
❖ 1922 – Mass production of insulin by Eli Lilly and company in collaboration with the University of Toronto in North America
❖ 1944 – Development of standard insulin syringe
❖ 1955 – Introduction of oral drugs for lowering blood glucose levels
❖ 1959 – Recognition of type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes
❖ 1966 – First pancreas transplantation at University of Manitoba
❖ 1970 – Development of blood glucose meters and insulin pumps
❖ 1983 – Introduction of biosynthetic human insulin
❖ 1986 – Introduction of insulin pen delivery system

A.2.2 Classification and diagnosis of diabetes

As knowledge of diabetes continued to develop, an International Expert Committee was formed in 1995 to revise the nomenclature, diagnostic criteria and classification of diabetes developed by the National Diabetes Data Group (NDDG) in 1979 [1]. The salient features of the changes in NDDG/WHO classification are as below:
A.2.2.1 Definition and description of diabetes:

- Diabetes mellitus is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels.

- The committee recommended the recognition of two major forms of diabetes, which they termed type 1 and type 2 diabetes to include evidence that diabetes mellitus was an etiologically and clinically heterogeneous group of disorders that share hyperglycaemia in common. Broadly, the committee proposed classification of diabetes based on aetiology into four types, viz., type 1 diabetes (β-cell destruction, usually leading to absolute insulin deficiency), type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance), Gestational diabetes mellitus (GDM), and other specific types that include 8 sub-types. The class termed ‘malnutrition-based related diabetes has been eliminated.

- Patients with any form of diabetes may require insulin treatment at some stage of their disease. Such use of insulin does not, of itself, classify the patient.

- The terms insulin-dependent diabetes mellitus and non–insulin-dependent diabetes mellitus and their acronyms, IDDM and NIDDM, are eliminated. The terms type 1 and type 2 diabetes are retained, with Arabic numerals being used rather than roman numerals.

- Type 2 diabetes includes the most prevalent form of diabetes, which results from insulin resistance with an insulin secretory defect.

- The stage termed impaired glucose tolerance (IGT) has been retained. The analogous intermediate stage of fasting glucose is named impaired fasting glucose (IFG). Selective rather than universal screening for glucose intolerance in pregnancy is now recommended.

- The degree of hyperglycaemia may change over time and reflects the severity of underlying metabolic process and its treatment more than the nature of the process itself (Figure A.6).
For the clinician and patient, it is less important to label the particular type of diabetes than is to understand the pathogenesis of the hyperglycaemia and to treat it effectively.

<table>
<thead>
<tr>
<th>Types</th>
<th>Normoglycemia</th>
<th>Hyperglycemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal glucose regulation</td>
<td>Impaired glucose tolerance or Impaired fasting glucose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1 *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other Specific types **</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational Diabetes **</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Even after presenting in ketoacidosis, these patients can briefly return to normoglycemia without requiring continuous therapy (i.e., “honeymoon” remission); ** in rare instances, patients in these categories (e.g., Vacor toxicity, Type 1 diabetes presenting in pregnancy) may require insulin for survival. (Table reproduced from Diabetes Care 2003; Volume 26; Supplement 1:S5-S20).

Figure A.6. Disorders of glycaemia: Etiologic types and stages.

A.2.2.2 Impaired glucose tolerance and impaired fasting glucose:

The terms impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) that refer to a metabolic stage intermediate between normal glucose homeostasis and diabetes, now referred to as pre-diabetes, are defined as described below:

A.2.2.2.1 Criteria for the diagnosis of diabetes mellitus in epidemiological studies:

- Symptoms of diabetes plus casual plasma glucose concentration ≥ 200 mg/dL (11.1 mmol/L). Casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydypsia, and unexplained weight loss

  Or

- Fasting plasma glucose (FPG) ≥ 126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 h
Or

- 2-h post-load glucose (2-h PG) ≥ 200 mg/dL (11.1 mmol/L) during an OGTT. The test should be performed as described by WHO [9], using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water

In the absence of unequivocal hyperglycaemia with acute metabolic decompensation, these criteria should be confirmed by repeat testing on a different day. The third measure (OGTT) is not recommended for routine clinical use

A.2.2.2.2 Criteria for diagnosis of intermediate group of subjects whose FPG levels / 2-h PG values in OGTT are below those mentioned in epidemiological studies, but are too high to be considered altogether normal:

⇒ FPG < 110 mg/dL (6.1 mmol/L) = normal fasting glucose
⇒ FPG ≥ 110 mg/dL (6.1 mmol/L) and < 126 mg/dL (7.0 mmol/L) = IFG
⇒ FPG ≥ 126 mg/dL (7.0 mmol/L) = provisional diagnosis of diabetes (the diagnosis must be confirmed, as described above)

The corresponding OGTT values are as below:

⇒ 2-h PG < 140 mg/dL (7.8 mmol/L) = normal glucose tolerance
⇒ 2-h PG ≥ 140 mg/dL and < 200 mg/dL (11.1 mmol/L) = IGT
⇒ 2-h PG ≥ 200 mg/dL (11.1 mmol/L) = provisional diagnosis of diabetes (the diagnosis must be confirmed, as described above)

A.2.2.2.3 Criteria for testing of diabetes in asymptomatic, undiagnosed individuals:

⇒ Testing for diabetes should be considered in individuals at age 45 years and above, particularly in those with a BMI ≥ 25 kg/m²; if normal, it should be repeated at 3-year intervals
⇒ Testing should be considered at a younger age or be carried out more frequently in individuals who are overweight (BMI ≥ 25 kg/m²; this may not be correct for all ethnic groups) and have additional risk factors:
  • have a first-degree relative with diabetes
  • are habitually physically inactive
• are members of a high-risk ethnic population (e.g., African-American, Hispanic American, Native American, Asian American, Pacific Islander)
• have delivered a baby weighing > 9 lb or have been diagnosed with GDM
• are hypertensive (≥ 140/90)
• have an HDL cholesterol level ≤ 35 mg/dL (0.90 mmol/L) and/or a triglyceride level ≥ 250 mg/dL (2.82 mmol/L)
• have PCOS
• on previous testing, had IGT or IFG
• have a history of vascular disease

⇒ The OGTT or FPG test may be used to diagnose diabetes; however, in clinical settings the FPG test is greatly preferred because of ease of administration, convenience, acceptability to patients, and lower cost.

A.2.3 Physiology and anatomy of pancreas and insulin action

The pancreas is a vital organ for digestion and glucose homeostasis in higher organisms. Malfunction of the pancreas results in several debilitating diseases such as diabetes, pancreatitis, pancreatic cancer, etc. The pancreas possesses both exocrine and endocrine functions. The exocrine function, involved in the delivery of enzymes into the digestive tract, is carried out by the cells of acini that secrete various digestive juices (proteases, amylases, nucleases, etc.) and ductal cells that transport these enzymes to the intestine. Through its endocrine function, the pancreas secretes several hormones into the bloodstream to coordinate and regulate glucose utilization. The functional unit of endocrine pancreas is islet of Langerhans. Human pancreas has 1 to 2 million islets of Langerhans, each about 0.3 mm in diameter and organized around small capillaries into which its cells secrete their hormones. These islets are dispersed throughout the exocrine portion of the pancreas and are composed of four cell types: α, β, δ and PP cells, distinguished from one another by their morphologic and staining characteristics. The insulin-producing β-cells represent the majority of the endocrine cell population and lie in the middle of each islet whereas the sparser α, δ and PP cells secrete glucagon, somatostatin and a pancreatic polypeptide respectively and are found at the periphery of the islets. While insulin inhibits glucagon secretion, somatostatin inhibits the secretion of both insulin and glucagon. Most of the endogenous glucagon and insulin are cleared from the circulation by the liver [10]. Insulin exerts multiple effects in the cell (Figure A.7).
By convention, the basal state is the metabolic condition prevailing in the morning after an overnight (10-14 h) fast. In a normal person, the blood glucose concentration is narrowly controlled, usually between 80 and 90 mg/dL of blood after an overnight fast. Liver functions as an important blood glucose buffer system. After a meal, blood glucose and insulin levels increase and liver immediately stores as much as two thirds of the glucose absorbed from the gut in the form of glycogen. During the succeeding hours, when both blood glucose levels and rate of insulin secretion fall, liver releases the glucose back into the blood [1].

Figure A.7. Insulin action in the cell.
Insulin exerts multiple effects in the cell. Insulin action is mediated by the binding of insulin to its receptor, and the subsequent phosphorylation of the receptor and other substrates by the receptor tyrosine kinase

A.2.3.1 Insulin receptors, signalling and actions in normal and disease states
The first substrate (intracellular protein) of the insulin receptor, described by White et al., was cloned by Sun et al. [11,12] and named insulin receptor substrate-1 (IRS-1). Subsequently other IRS proteins (IRS-2, -3, -4) were cloned. These are phosphorylated upon insulin stimulation and have adaptor function between the insulin receptor and other cellular substrates such as the phosphatidylinositol 3-kinase (PI 3-kinase) (Figure A.8) [13,14,15]. The contribution of IRS-1 and IRS-2 to insulin resistance and diabetes was tested by knocking of the respective genes in mice. IRS-1 knockout mice were insulin resistant but not hyperglycaemia [16]. IRS-2-deficient mice were found to be severely hyperglycaemia due to insulin resistance and
insufficient insulin secretion because of reduced β-cell mass and revealed many similarities to type 2 diabetes in man outlining the role of IRS proteins in the development of cellular insulin resistance and β-cell function. [17].

Figure A.8. Molecular mechanism of insulin-stimulated transport.

The insulin-dependent glucose transporter 4 (GLUT4) is translocated by a phosphatidylinositol 3-kinase (PI 3K)-dependent pathway including PKB/AKT and PKC stimulation downstream of PI3K. PI3,4,5P -phosphatidylinositol 3,4,5-phosphate; PDK -phosphatidylinositol (3,4,5)-phosphate-dependent kinase; IRS -insulin receptor substrate.

### A.2.3.2 Glucose transporters

Glucose transporters represent a large family of proteins encompassing GLUT1 to GLUT5 that facilitate glucose transport into the cells across the plasma membrane. These transporters have different biochemical properties and the genes encoding these proteins are differentially expressed and regulated in various tissues (table A.2). GLUT4 is the major insulin-sensitive transporter in muscle and adipose tissue, the major sites for postprandial glucose disposal, where it is present in intracellular vesicles that are translocated to the plasma membrane in response to insulin. The islet cell that produces insulin as a direct response to the degree of hyperglycaemia is freely permeable to glucose via the GLUT 2 transporter, and the glucose is phosphorylated by the high-K$_m$ glucokinase. Hence, the blood glucose levels determine the flux through glycolysis, the citric acid cycle, and the generation of ATP. Increase in ATP concentration inhibits the ATP-sensitive K$^+$ channels, causing depolarization of the β-
cell membrane, which increases $\text{Ca}^{2+}$ influx via voltage-sensitive $\text{Ca}^{2+}$ channels, stimulating exocytosis of insulin (Figure A.9). Thus, the concentration of insulin in the blood parallels that of the blood glucose.

Table A.2. Glucose transporters.

<table>
<thead>
<tr>
<th>Tissue Location</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Facilitative bidirectional transporters</strong></td>
<td></td>
</tr>
<tr>
<td>GLUT 1 Brain, kidney, colon, placenta, erythrocyte</td>
<td>Uptake of glucose</td>
</tr>
<tr>
<td>GLUT 2 Liver, pancreatic β cell, small intestine, kidney</td>
<td>Rapid uptake and release of glucose</td>
</tr>
<tr>
<td>GLUT 3 Brain, kidney, placenta</td>
<td>Uptake of glucose</td>
</tr>
<tr>
<td>GLUT 4 Heart and skeletal muscle, adipose tissue</td>
<td>Insulin-stimulated uptake of glucose</td>
</tr>
<tr>
<td>GLUT 5 Small intestine</td>
<td>Absorption of glucose</td>
</tr>
<tr>
<td><strong>Sodium-dependent unidirectional transporter</strong></td>
<td></td>
</tr>
<tr>
<td>SGLT 1 Small intestine and kidney</td>
<td>Active uptake of glucose from lumen of intestine and reabsorption of glucose in proximal tubule of kidney against a concentration gradient</td>
</tr>
</tbody>
</table>

Figure A.9. Schematic representation of the mechanisms controlling the initiation and some amplification pathways of insulin secretion in the pancreatic β-cell.
A.2.3.3 Glucose metabolism and importance of blood glucose regulation

Glucose is the only nutrient that is normally utilized by the brain, retina, and germinal epithelium of the gonads in sufficient quantities to supply them optimally with their required energy. To fulfill this action, it is important to maintain blood glucose levels at a sufficiently high level. Most of the glucose formed by gluconeogenesis during the interdigestive period is used for metabolism in the brain and pancreas does not secrete insulin during this time; else, the scant supplies of glucose that are available would all go into the muscles and other peripheral tissues, leaving the brain without a nutritive source. In brain, liver, kidney, intestine and placenta, glucose utilization is insulin independent; in adipose tissue, skeletal and heart muscle, glucose uptake depends on insulin. However, it is equally important that the blood glucose levels not rise too high for three reasons: First, glucose exerts a large amount of osmotic pressure in the extracellular fluid, and if the glucose concentration rises to excessive values, this can cause considerable cellular dehydration. Second, an excessively high blood glucose concentration causes loss of glucose in the urine. Third, this causes osmotic diuresis by the kidneys, which can deplete the body of its fluids and electrolytes [].

In severe hypoglycaemia, a direct of low blood glucose on the hypothalamus stimulates the sympathetic nervous system, and in turn adrenal glands secrete epinephrine that causes release of glucose from the liver to combat the severe hypoglycaemia. Over a period of hours and days, both growth hormone and cortisol are secreted in response to prolonged hypoglycaemia, and both of them decrease the rate of glucose utilization by most cells of the body, converting instead to greater amounts of fat utilization, there by helping blood glucose concentration return toward normal [].

Other hormones that affect blood glucose []:

Growth hormone and corticotrophin (ACTH) secreted by anterior pituitary gland tend to elevate the blood glucose, there by antagonizing the action of insulin. Growth hormone, the secretion of which is stimulated by hypoglycaemia, decreases glucose uptake in tissues such as muscle. Some of this effect may not be direct; since it mobilizes free fatty acids from adipose tissue which themselves inhibit glucose utilization. Chronic administration of growth hormone leads to diabetes. By producing
hyperglycaemia, it stimulates secretion of insulin, causing β-cell exhaustion in due course.

Administration of glucocorticoids, secreted by adrenal cortex, causes increased gluconeogenesis. This is a result of increased protein catabolism in the tissues, increased uptake of amino acids, and increased activity of aminotransferases and other enzymes concerned with gluconeogenesis in the liver. In addition, they inhibit the utilization of glucose in extrahepatic tissues. In all these actions, glucocorticoids act in an antagonistic manner to insulin.

Adrenal medulla secretes epinephrine in response to stressful stimuli, such as excitement, haemorrhage, hypoxia, and hypoglycaemia. This leads to glycogenolysis in liver muscle owing to stimulation of phosphorylase via generation of cAMP. In muscle, as a result of the absence of glucose-6-phosphatase, glycogenolysis ensues with the formation of lactate, whereas in liver, glucose is the main product leading to increase in blood glucose.

Thyroid hormone also affects blood glucose. In humans, the fasting blood glucose is elevated in hyperthyroid patients and decreased in patients with hypothyroidism. While the former utilize glucose at a normal or increased rate, the latter have a decreased ability to utilize glucose and are less sensitive to insulin compared with normal or hyperthyroid subjects.

**A.2.4 Pathophysiology of type 2 diabetes**

The development of type 2 diabetes is characterized by progression from normal glucose tolerance to impaired glucose tolerance (IGT) to diabetes. The pathophysiology of type 2 diabetes encompasses progressive pancreatic β-cell dysfunction and insulin resistance in all major target tissues, such as skeletal muscle, kidney, liver and adipose tissue. Current diagnostic criteria to ascertain type 2 diabetes are described in section A.2.2 above. Most of the newly diagnosed type 2 diabetic subjects already suffer from “late complications of diabetes” at the time of diagnosis [19], highlighting the fact that it represents only the “tip of the iceberg” of long existing metabolic disturbances with deleterious effects on the vascular system, tissues and organs [20]. Pathogenesis of type 2 diabetes is schematically represented in Figure A.10 [21]. The United Kingdom Prospective Diabetes Study (UKPDS) demonstrated that non-pharmacological treatments, such as reduced caloric intake and increased physical activity, as the primary option is sufficient only in 25% of patients.
with a 3-year history (after the diagnosis) of diabetes [22]. Though their efficacy has been demonstrated in many studies [23,24], the actual number of patients sufficiently treated without pharmacological agents is comparatively low. As the disease duration advanced with associated progressive deterioration in β-cell function [25], the percentage of responders to non-pharmacological treatments fell down to 10% after 9 years [19]. These data clearly indicate that pharmacological treatment is required in the vast majority of type 2 diabetic population [].

![Diagram of type 2 diabetes pathogenesis](image)

Figure A.10. Pathogenesis of type 2 diabetes.

The evolution from normal glucose tolerance to clinically overt type 2 diabetes is associated with progressive insulin resistance and β-cell insulin secretory deficiency.

### A.2.4.1 Insulin resistance

Skeletal muscle, liver and adipose tissue are some of the major metabolic sites for insulin action. Insulin resistance is defined as a steady-state plasma glucose level that is higher than would be expected for the prevailing plasma insulin. Insulin resistance is commonly associated with obesity and type 2 diabetes. The term is often used synonymously with impaired insulin-stimulated glucose disposal, as measured with the hyperinsulinaemic-euglycaemic clamp technique [26-27,28]. Insulin-induced inhibition of lipolysis is an exquisitely sensitive measure, with EC_{50} values that fall in the physiological insulinaemic range [29]. Overproduction of nonesterified fatty acids and over-expression of tumour necrosis factor-alpha (TNFα) by adipocytes have been implicated in the pathogenesis of insulin resistance [30,31]. Mechanistically, insulin
resistance is a complex syndrome that involves a number of molecular defects at different levels along the insulin signalling cascade [32]. Excessive basal glucose production in the presence of fasting hyperinsulinaemia is the key feature of type 2 diabetes [33,34]. Defective suppression of endogenous glucose production by normal or elevated insulin levels is observed in type 2 diabetes [35]. Involvement of insulin resistance of glucose production in the pathogenesis of type 2 diabetes becomes evident with these observations (Figure A.11).

![Image](image-url)  
**Figure A.11. Insulin resistance.**

### A.2.4.2 β-cell dysfunction [11]

The pancreatic β-cells secrete insulin in response to glucose stimulation through a series of transmembrane electrical reactions (Figure A.9). It has been suggested that the final common pathway responsible for the development of type 2 diabetes is the failure of the pancreatic β-cell to compensate for insulin resistance. An absolute decompensation in β-cell function is seen during transition from IGT to diabetes (Figure A.12). It is not clear whether the progressive β-cell dysfunction results from pre-programmed genetic abnormalities, acquired defects, or combination of both. A loss of β-cell differentiation can be found in the early stage of type 2 diabetes [36]. The β-cell mass is lower in type 2 diabetic patients compared with obese control subjects, but the reduction is modest and does not completely explain the hyperglycaemia.
Thus the functional loss (i.e. impaired glucose-mediated insulin secretion) exceeds the expected impact of a 20-50% loss of β-cell mass, reported in autopsy studies of type 2 diabetes patients [].

![Figure A.12. Insulin resistance and type 2 diabetes.](image)

**A.2.4.3 Hepatic glucose output**

Basal rates of hepatic glucose production (HGP) are variably increased in patients with type 2 diabetes but are normal in their normoglycaemic first-degree relatives. The ability of insulin to suppress HGP also is normal in first-degree relatives, suggesting that dysregulated HGP is probably acquired late in the pathogenesis. The increased endogenous glucose output, triggered by an increased flux of gluconeogenic precursors (such as glycerol, FFAs, and alanine), is a result, at least in part, of hepatic insulin resistance. The diabetic state increases fat utilization as alternative fuel; products of the resultant lipolysis and fatty acid oxidation, such as acetyl-coenzyme A, are potent stimuli for hepatic gluconeogenesis. There are no abnormalities in the activities of the principal enzymes of gluconeogenesis such as phosphoenolpyruvate carboxykinase, fructose 1,6-biphosphatase, and glucose-6-phosphatase in type 2 diabetes. Hepatic insulin resistance is associated with a decrease in glucokinase activity, which catalyzes a crucial step in hepatic glucose metabolism. The liver in type 2 diabetes thus appears to be programmed to overproduce and underutilize glucose.
A.2.5 Complications of diabetes [37]

Diabetes (both type 1 and type 2) is a debilitating and true multisystem disease. In addition to creating day-to-day challenges in glycaemic control, it causes gradual breakdown of vital bodily functions leading to disabling, and in some cases, life-threatening complications. In both types of diabetes, abnormally high levels of blood glucose, as well as other metabolites, damage both the small and large blood vessels, producing microvascular and macrovascular complications respectively, that affect almost each and every organ of the body.

A.2.5.1 Microvascular complications

The term ‘microvascular complications’ encompasses the effects of diabetes on the small blood vessels throughout the body that lead to severe damage to the eye, kidney and nervous system. Each of these complications has distinct pathophysiological features and may require distinct therapeutic approaches.

A.2.5.1.1 Ocular related complications

‘Retinopathy’, the most common form of diabetic eye disease that damages the retina, develops when small blood vessels that supply the retina with oxygen and other nutrients are damaged. Virtually all people with Type 1 diabetes have retinal damage, with 30 percent having the most severe form. Approximately, 80% of the type 2 diabetics who take insulin have retinopathy after 15 years and 10 to 15 percent have proliferative retinopathy.

A.2.5.1.2 Kidney related complications

Nephropathy occurs as a result of damage to kidneys that affects their functional ability. The damage is usually a silent process, gradually progress over a long period and manifests itself only when <25% of renal function remains active. After 5 years of diagnosis of diabetes, approximately, 10% of type 2 diabetics develop clinically detectable proteinuria and 20% show signs of renal damage after 20 years.

A.2.5.1.3 Nervous system related complications

Diabetes affects many parts of the nervous system. The damage is partly due to the effect on the small blood vessels and also probably due to direct effect on the nerve tissue itself, resulting in the damage of both the peripheral nerves (nerves involved in
sensation and movement) – *Peripheral neuropathy*, and the autonomic nerves (nerves that control many internal functions, such as heart rate, gastric motility, bladder function, normal sexual response, etc.) – *Autonomic neuropathy*. While peripheral neuropathy causes pain and loss of sensation, contributing to the increased risk for ulceration, limb infection and amputation, autonomic neuropathy may lead to heart arrhythmias, poor control of blood pressure, and digestive and sexual dysfunction.

**A.2.5.1.4 Oral complications of diabetes**

Oral complications of diabetes are extremely common, difficult to treat within poorly controlled patients and reduce the quality of life. They include mucosal infections, salivary gland dysfunction leading to difficulty swallowing and speaking.

**A.2.5.2 Macrovascular complications**

Macrovascular complications (damage to the large blood vessels) is the most common cause of death in type 1 and type 2 diabetic patients and diabetes is a major cause of cardiac, cardiovascular disease (CVD) and peripheral vascular damage. In diabetes, the supply of nutrients and oxygen to tissues is impaired by the combination of peripheral vascular disease of the large blood vessels (atherosclerosis) and by the microvascular damage to the small blood vessels and capillaries, which nourish the same area. Individuals with diabetes also have some damage to the heart muscle (cardiomyopathy) and to the nerves that supply the heart (cardiac autonomic neuropathy) as a result of the abnormal metabolism present in this disease. A diabetic patient with existing or incipient macrovascular diseases requires multiple modifications of lifestyle and diet, as well as a poly-pharmaceutical approach to address the needs for glucose control, optimization of lipid levels and blood pressure, and other disease risk factors.

**A.2.6 Current treatment options for the therapeutic management of type 2 diabetes**

Currently available oral antihyperglycaemia agents (OHAs) for type 2 diabetes are presented in [Figure A.13](#) and [Figure A.14](#) and [Table A.3](#) and [Table A.4](#) \[^{[38]}\].
Figure A.13. Major target organs and actions of orally administered antihyperglycemic agents in type 2 diabetes mellitus.

TZD = thiazolidinedione; FFA = free fatty acid; AGI = α-glucosidase inhibitor.

Table A.3. Food and Drug Administration-approved Indications for oral Antidiabetic Agents

<table>
<thead>
<tr>
<th>Drug</th>
<th>FDA-approved Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfonylureas</td>
<td>Monotherapy or with metformin, alpha-glucosidase inhibitors, thiazolidinediones, or insulin</td>
</tr>
<tr>
<td>Biguanides (Metformin)</td>
<td>Monotherapy or with sulfonylurea or insulin</td>
</tr>
<tr>
<td>Alpha-glucosidase inhibitors</td>
<td>Monotherapy or with sulfonylurea, metformin, or insulin (Miglitol only approved for use with sulfonylurea)</td>
</tr>
<tr>
<td>Thiazolidinediones</td>
<td>Monotherapy or with sulfonylurea, metformin, or insulin (Rosiglitazone not approved for use with insulin)</td>
</tr>
<tr>
<td>Nonsulfonylurea secretagogues</td>
<td>Monotherapy or with metformin</td>
</tr>
<tr>
<td>Glucovance® (Glyburide + Metformin)</td>
<td>Monotherapy or in combination with a thiazolidinedione</td>
</tr>
<tr>
<td>Metaglip® (Glipizide + Metformin)</td>
<td>Monotherapy</td>
</tr>
<tr>
<td>Avandamet® (Rosiglitazone + Metformin)</td>
<td>Monotherapy</td>
</tr>
</tbody>
</table>
Figure A.14. Insulin secretagogues mimic glucose to close adenosine triphosphate sensitive potassium channels (kir6.2) and stimulate insulin secretion. (A) shows the ion channel in a resting pancreatic β cell. (B) shows the action of insulin secretagogues on the cell.
### Table A.4. Orally administered antihyperglycemic agents (OHAs) for the treatment of diabetes.

<table>
<thead>
<tr>
<th>Drug class</th>
<th>Mechanism of action</th>
<th>Primary site of action</th>
<th>Dosage</th>
<th>Decrease in HbA1c concentration*</th>
<th>Main side effects</th>
<th>Drug interactions</th>
<th>Contraindications</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Glucosidase inhibitor</td>
<td>Delays intestinal carbohydrate absorption</td>
<td>Small intestines</td>
<td>25 mg once daily, titrated to 100 mg 3 times daily</td>
<td>0.5%–1.0%</td>
<td>Gastrointestinal</td>
<td>-</td>
<td>Irritable bowel syndrome, severe kidney or liver dysfunction</td>
</tr>
<tr>
<td>(acarbose)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biguanide†</td>
<td>Increases liver and muscle insulin sensitivity; decreases hepatic glucose production</td>
<td>Liver, peripheral tissue</td>
<td>500 mg once daily, titrated to 1000 mg twice daily</td>
<td>1.0%–1.5%</td>
<td>Gastrointestinal, lactic acidosis (rare)</td>
<td>Alcohol († risk of lactic acidosis)</td>
<td>Moderate to severe liver or cardiac dysfunction, mild renal dysfunction‡</td>
</tr>
<tr>
<td>(metformin)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin secretagogue</td>
<td>Increases insulin secretion</td>
<td>Pancreas</td>
<td>Gliclazide: 40–160 mg twice daily (MR form) Glimepiride: 1–8 mg once daily Glyburide: ≤ 5 mg once daily, titrated to &gt; 5 mg twice daily</td>
<td>1.0%–1.5%</td>
<td>Hypoglycaemia, weight gain</td>
<td>Many</td>
<td>Moderate to severe liver dysfunction; adjust dose in the presence of severe kidney dysfunction. Avoid use of glyburide in elderly patients or patients with kidney dysfunction.</td>
</tr>
<tr>
<td>Sulfonylureas</td>
<td></td>
<td></td>
<td>Repaglinide: 0.5–4 mg 3 times daily Nateglinide: 60–120 mg 3 times daily</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(gliclazide, glimepiride, glyburide)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-sulfonylureas</td>
<td>Acute increase of insulin secretion</td>
<td>Peripheral tissue, liver</td>
<td>Rosiglitazone: 2–8 mg once daily Pioglitazone: 15–45 mg once daily</td>
<td>1.0%–1.5%</td>
<td>Weight gain, edema, anemia, pulmonary edema, CHF</td>
<td>† effect by CYP 450 3A4 inhibitors; † effect by CYP 450 3A4 inducers</td>
<td>Severe liver or kidney dysfunction; avoid concomitant use of repaglinide with gemfibrozil</td>
</tr>
<tr>
<td>(repaglinide, nateglinide)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin sensitizer or</td>
<td>Increases adipose and muscle insulin sensitivity</td>
<td>Peripheral tissue, liver</td>
<td></td>
<td>1.0%–1.5%</td>
<td>Weight gain, edema, anemia, pulmonary edema, CHF</td>
<td>† effect by CYP 450 2C8 and 2C9 inhibitor- Gemfibrozil</td>
<td>Severe liver dysfunction, NYHA class II-IV CHF</td>
</tr>
<tr>
<td>thiazolidinediones§</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(rosiglitazone, pioglitazone)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal lipase inhibitor¶</td>
<td>Decreases intestinal fat absorption (weight loss)</td>
<td>Intestinal tract</td>
<td>120 mg 3 times daily</td>
<td>0.3%–0.9%</td>
<td>Gastrointestinal, reduced absorption of fat-soluble vitamins</td>
<td>Malabsorption syndrome, cholestasis</td>
<td></td>
</tr>
<tr>
<td>(orlistat)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** MR = modified release, CHF = congestive heart failure, NYHA = New York Heart Association, *Indicated average decreases in hemoglobin A1c concentrations after 3–6 months of monotherapy, †Preferred primary agent for overweight patients, ‡ Use with caution or avoid in the presence of any elevation in serum creatinine levels, § 6–12 weeks are required to achieve the full glucose-lowering effect, ¶ Suitable for obese patients only.
A.2.6.1 Combinations to be avoided

- Sulfonylurea and nonsulfonylurea insulin secretagogue
- Insulin secretagogue and pre-prandial insulin
- Thiazolidinedione and insulin

Combinations of sub-maximal doses of different classes of OHAs may be equally effective as or more effective than maximum dose of monotherapy in improving glucose control with fewer adverse effects.

A.2.7 Novel targets for the treatment of diabetes

Irrespective of the underlying cause of diabetes, goal of any kind of treatment is directed at normalization of blood glucose. For normal metabolism insulin must be released from the pancreas in an exquisitely exact amount, at the right time and in a right pattern. The normal pancreas also senses the fasting and fed state as well as the energy content of the meals eaten. At any point of time glucose homeostasis is maintained by a balance between insulin secretion and insulin action. The robustness of the physiological system existing in non-diabetic people takes care of the alterations in any of these parameters. None of the available pharmacological agents can either take the place of this exquisite sensing capacity or restore the pattern of insulin kinetics precisely. This is evident from the presence of so many compounds to treat type 2 diabetes.

In light of recent progress made in understanding insulin resistance and pancreatic β-cell dysfunction, the two major defects in type 2 diabetes, several novel therapeutic approaches have been evolved to address both defects. Some of the important targets are presented in Table A.5 \(^{39,40}\). All these targets focus on improving insulin sensitivity and augmenting glucose-dependent insulin secretion.

Most of the novel targets mentioned in Table A.5 are in early stages of development and some of them even require a proof of concept in human/type 2 diabetic subjects.
In a few of these targets, GLP-1 receptor agonists and DPP-IV inhibitors in particular, have been extensively studied both in preclinical and clinical phases and are showing lot of promise. The present work is based on DPP-IV target and attempts have been made to provide preclinical evidence to the therapeutic utility of a DPP-IV inhibitor in the management of type 2 diabetes.

Table A.5. Molecular targets in type 2 diabetes.

<table>
<thead>
<tr>
<th>Mechanism of action</th>
<th>Target</th>
<th>Human proof of concept</th>
<th>Phase of development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic glucose production</td>
<td>Glucagon receptor</td>
<td>Yes*</td>
<td>Phase II?</td>
</tr>
<tr>
<td></td>
<td>Glycogen phosphorylase</td>
<td>Yes</td>
<td>Phase II?</td>
</tr>
<tr>
<td>Glucose-stimulated insulin secretion</td>
<td>GLP-1 receptor</td>
<td>Yes</td>
<td>Launched</td>
</tr>
<tr>
<td></td>
<td>DP-IV</td>
<td>Yes</td>
<td>Pre-registration</td>
</tr>
<tr>
<td>Insulin signaling</td>
<td>Insulin receptor</td>
<td>No</td>
<td>Preclinical</td>
</tr>
<tr>
<td></td>
<td>PTP-1B</td>
<td>Yes</td>
<td>Phase I</td>
</tr>
<tr>
<td></td>
<td>GSK-3</td>
<td>No</td>
<td>Preclinical</td>
</tr>
<tr>
<td></td>
<td>SHIP-2</td>
<td>No</td>
<td>Preclinical</td>
</tr>
<tr>
<td></td>
<td>1kB kinase</td>
<td>No</td>
<td>Preclinical</td>
</tr>
<tr>
<td></td>
<td>Protein kinase C-0</td>
<td>No</td>
<td>Preclinical</td>
</tr>
<tr>
<td></td>
<td>PPARγ</td>
<td>Yes</td>
<td>Launched</td>
</tr>
</tbody>
</table>

PTP-1B, Protein tyrosine phosphatase 1B; GSK-3, glycogen synthase kinase-3; SHIP-2, SH2 domain containing inositol-5- phosphatase type 2.

* Proof of concept demonstrated in healthy subjects, but not patients with type 2 diabetes.

The next section gives the rationale behind the selection of inhibitors of DPP-IV enzyme as the target for studying antidiabetic activity, along with pharmacology and potential of DPP-IV inhibitors for treatment of diabetes.
A.3 DPP-IV INHIBITORS

Unlike Type 1 diabetes, people with type 2 diabetes might make healthy, even high, levels of insulin, but there is a decrease in insulin action at insulin-sensitive tissues. Thus, the control of glucose levels in the blood is impaired. This resistance to insulin is often caused by obesity. Approximately 90–95% of people with diabetes have type 2 diabetes \[^4\]. Although type 2 diabetes is associated typically with older people, it has become much more prevalent among children and young adults, in line with the alarming rise of obesity in the general population. Currently available pharmacological treatments for type 2 diabetes are not satisfactory in various aspects (refer to Table A.3). Sulfonylureas have the drawback of causing hypoglycaemia and weight gain \[^42\]. These drugs are contraindicated in patients with liver dysfunction as they are metabolized in liver. Thiazolidinediones cause increase in body weight, oedema, anaemia, pulmonary oedema and congestive heart failure \[^43\]. However, the weight gain is more from peripheral than visceral fat \[^44\]. Islet dysfunction is one of the major phenomena in type 2 diabetes and an ideal treatment should normalize this condition and prevent weight gain.

With diabetes becoming the major cause of peripheral neuropathy afflicting some 20–30% of type 2 diabetics with no proper treatment other than strict control of blood glucose levels, endocrinology research over the past decade has focused on the development of a new therapeutic strategy for type 2 diabetes based on the insulinotropic actions of endogenous peptides. The gastrointestinal tract produces and harbours a number of biologically active peptides, some of which are true hormones produced in the gastrointestinal endocrine cells and released into the circulation following ingestion of nutrients. Other peptides are neuropeptides, \textit{ie}, they are produced in the autonomic nerves within the gastrointestinal tract (Table A.6). Several of these gut peptides have the ability to stimulate insulin secretion, and may be important for the regulation of a normal insulin secretion, particularly after nutrient ingestion. Since defective insulin secretion is a main cause of type 2 diabetes, the gut peptides having insulinotropic ability have been explored for the treatment of this disease.
Gut hormones that are released into the circulation following meal ingestion and stimulate insulin secretion postprandially are called *incretins*. The *incretins*, glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP) are involved in the stimulation of pancreatic β-cell. Incretin concept came into focus with the observation that oral glucose administration produced greater insulin release compared with that of intravenous administration, leading to a hypothesis that agents which stimulate insulin release to a greater extent when nutrients are ingested *via* enteral route than parenteral route would become potent insulin secretagogues \[45,46\]. Both the incretins are released in response to ingested food and augment insulin secretion \[45,47,48,49,50,51\]. GLP-1 is considered as one of the potential alternative treatments for type 2 diabetes due to its ability to stimulate insulin secretion in a glucose-dependent manner, inhibit glucagon release and delay gastric emptying. These effects combine in decreasing the circulating glucose \[51\]. The biological effects
of GLP-1 are presented in Figure A.15. GLP-1 is considered to be the most important incretin involved in the enteroinsular axis (a signalling pathway between gut and pancreatic islets that amplify the insulin response to absorbed nutrients) and inhibits glucagon secretion, hepatic glucose production and food intake, delays gastric emptying and promotes satiety, and has tropic effects on pancreatic β cells [52,53]. These actions of GLP-1 make it a good candidate for the treatment of metabolic disturbances in type 2 diabetes [54]. However, the biologically active form of GLP-1 has an extremely short half-life of about one minute and is rapidly hydrolyzed by DPP-IV enzyme.

![Figure A.15. Biological effects of GLP-1 in humans.](image)

The extremely short half-life of these hormones [55] following secretion makes them unattractive for chronic therapy of type 2 diabetes. Dipeptidyl peptidase-IV (DPP-IV; EC 3.4.14.5) is the enzyme responsible for the rapid degradation of GLP-1 (by proteolytic cleavage to an inactive metabolite) and inhibition of this enzyme is considered as one of the approaches to increase the circulating half-life of the hormone (Figure A.16). DPP-IV, a highly specific aminopeptidase, is widely distributed on the surface of various types of cells, particularly in the liver, kidney and small intestine and is present in soluble form in plasma. It is also expressed in pancreas, lung, testis, T-cells and central nervous system. The expression of DPP-IV in endothelial cells mainly contributes to its role in carbohydrate metabolism [56,57]. DPP-IV knockout mice and Fischer 344 rats with deactivated DPP-IV have shown improved glucose tolerance and protection from diet-induced obesity [58,59].
DPP-IV enzyme acts by cleaving off dipeptides from the amino terminus of peptides with preferentially proline or alanine at the penultimate position, resulting in the regulation of activities of various peptides []. Of prime importance is the degradation of GLP-1, one of the well-characterized physiological and pharmacological substrates of the enzyme. Inhibition of circulating DPP-IV activity with specific DPP-IV inhibitors enhanced GLP-1 half-life, and in turn, insulin secretion in various preclinical studies of impaired glucose tolerance [60,61,62]. A comparison of these two strategies is presented in Table A.7 [].

Table A.7. Comparison of the two strategies using GLP-1-based therapy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GLP-1 analogues</th>
<th>DPP-IV inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Route of administration</td>
<td>Parenteral (sc)</td>
<td>Oral</td>
</tr>
<tr>
<td>Pharmacokinetics</td>
<td>Short-acting to very long-acting</td>
<td>Medium-acting (few hours to 24 h)</td>
</tr>
<tr>
<td></td>
<td>(hours to days)</td>
<td></td>
</tr>
<tr>
<td>Mechanism of action</td>
<td>Through GLP-1 receptors</td>
<td>Through preventing degradation of GLP-1 and other peptides</td>
</tr>
<tr>
<td>Adverse events</td>
<td>Nausea</td>
<td>None or minor</td>
</tr>
<tr>
<td>Efficiency</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>Risk of hypoglycaemia</td>
<td>Very low</td>
<td></td>
</tr>
<tr>
<td>Tolerability</td>
<td>Good tolerability</td>
<td></td>
</tr>
</tbody>
</table>
A.3.1 Structural properties and expression of DPP-IV

DPP-IV is a serine-type peptidase and was isolated from bacteria and eukaryotes. It was first reported in 1966 as glycyl-prolyl-β-naphthylamidase and subsequently was named dipeptidyl aminopeptidase IV, post-proline dipeptidyl aminopeptidase, and X-Pro dipeptidyl aminopeptidase. DPP-IV belongs to the proteolytic enzymes or peptidases. The current name ‘dipeptidyl-peptidase IV’ was recommended by the Enzyme Commission as per classification of enzymes based on their catalytic activity. Class 3 contains hydrolases, and the peptides that form enzyme sub-class 3.4 are divided into 13 sub-classes. Sub-class 3.4.14 contains dipeptidyl-peptidases and DPP-IV was given the identification number EC 3.4.14.5.

A.3.1.1 Expression of DPP-IV

DPP-IV enzyme is widely distributed throughout the body in mammalian tissues, mainly on epithelial and endothelial cell surfaces, on fibroblasts and lymphocytes, with its activity per gram tissue being highest in the kidney (in the kidney cortex on the glomerular basement membrane and the proximal convoluted tubules), followed by the lung, adrenal gland, jejunum, liver, parotid gland, spleen and testis. It is also expressed in epithelial cells of the pancreatic duct. In blood vessels, DPP-IV is expressed in the venous part of the capillary bed and on the endothelial cells of the blood vessels throughout the body and also circulates as a soluble enzyme.

A.3.1.2 Molecular aspects and physiology of DPP-IV

The primary structure of the enzyme was first determined by cloning and sequencing of the cDNA for rat DPP-IV. CD26, a surface marker of thymocytes and T lymphocytes, rat liver membrane glycoprotein gp110 and the murine thymocyte-activating molecule were found to be identical to DPP-IV. DPP-IV is a glycoprotein consisting of 766 and 767 amino acids in humans and rats respectively, with an 85% homology between the two sequences. The gene encoding for the enzyme is located on the long arm of chromosome 2 (2q24.3) and it contains 26 exons. Several transcription factors, such as NFκB or AP2, bind to the gene. DPP-IV is expressed on cell surfaces as a dimer with two units anchored through their N-terminal ends located close to each other extracellularly. The C-terminal, catalytic sites, of the two DPP-IV
enzymes located in the dimer form a small pocket thereby having localized high catalytic activity.

DPP-IV is a protease with the catalytic site in the C-terminal extracellular end of the sequence. It preferentially cleaves oligopeptides after the 2nd amino acid, releasing a dipeptide from oligopeptide. The active site of the enzyme resides in a small five-amino acid region centred around a serine residue in position 630; amino acid substitution in this region results in loss of catalytic activity. DPP-IV enzyme is widely spread with activity to cleave the two N-terminal amino acids of a number of biologically active peptides involved in various functions in endocrinology, gastroenterology and immunology.

The physiological function of DPP-IV is far from understood. Its distribution to organs having physiological barriers and involved in nutrition or secretion suggests that the enzyme is of physiological importance for defence and nutritional/digestive functions. DPP-IV may also be regarded as a proteolytic enzyme involved in the inactivation of bioactive peptides, particularly in relation to immunomodulation and glucose homeostasis. Another potential function of DPP-IV is a binding function, because a cystein-rich domain of the enzyme apart from the catalytic site binds preferentially to collagen, which may be of importance for its function. Hence, DPP-IV may function both as a protease and a binding protein.

**A.3.2 Role of DPP-IV in diabetes and glucose tolerance**

DPP-IV inactivates a number of peptides (GLP, GIP, glucagon, VIP, PACAP) involved in glucose homeostasis. DPP-IV is present in the endothelium of capillaries adjacent to the L cells secreting GLP-1, and a significant proportion of newly secreted GLP-1 is truncated even before it enters the systemic circulation [65]. GLP-1 administration to type 2 diabetic patients results in an improved glucose tolerance and decreased food intake. The plasma levels of GLP-1 transiently rise within 5 to 15 min after a meal. The major drawback of the therapeutic use of GLP-1 is its metabolic instability, because the half-life of intact GLP-1 in humans is 1 to 2 minutes [66], and truncated GLP-1 acts as an antagonist of GLP-1 receptor [67]. In the wake of these
developments, two strategies have evolved, the first one being development of DPP-IV resistant GLP-1 receptor agonists (GLP-1 mimetics) and the second one is the development of compounds that inhibit DPP-IV enzyme. DPP-IV-resistant analogues have been developed with prolonged biological activity and increased potency \textit{in vivo} []. The rationale for DPP-IV inhibition as a target for treatment of type 2 diabetes is therefore the inhibition of degradation of endogenously released GLP-1, resulting in the prolonged concentrations of the active form of GLP-1 and exploiting its efficient antidiabetic actions. The inhibition of DPP-IV will also increase the concentrations of active GIP, however this probably has only minor impact on metabolism in diabetes, since it has been demonstrated that the insulin response to GIP is strongly reduced in type 2 diabetes as well as in first degree relatives of patients with type 2 diabetes [,,68,69,70].

Improvement in glucose tolerance with \textit{in vivo} inhibition of DPP-IV is well established with experiments done in mice, rats, dogs, monkeys and pigs. Studies performed CD26–/– mice showed that DPP-IV contributes to blood glucose regulation by controlling the activity of GLP-1 as well as additional substrates [”]. After glucose challenge, the lack of DPP-IV expression leads to significantly faster blood glucose clearance, accompanied with increased insulin levels. No significant differences are detected in the fasting levels of glucose, insulin, or GLP-1 in these mice compared with DPP-IV-positive animals. After oral glucose, insulin levels were significantly lower in the DPP-IV-negative rats. Of the incretins, the GIP response was decreased by 50%, while the GLP-1 release and its insulinotropic activity remained unchanged compared with DPP-IV-positive rats. The role of DPP-IV mediated cleavage of neuropeptides and incretins on the stimulation of insulin secretion in \(\beta\)-cells is depicted in Figure A.17.

Several DPP-IV inhibitors, \textit{viz.} NVP DPP728, FE 999011, P32/98, BMS-477118 (Saxagliptin), MK-0431 (Sitagliptin) and LAF 237 (Vildagliptin) currently in various stages of preclinical and clinical development showed sustained improvements in glucose tolerance and delay in the progression from impaired glucose tolerance to overt diabetes after long-term DPP-IV inhibition in diabetogenic rats and mice [47,53,59,61,62,63,73,74,75].
The possible influence of DPP-IV mediated cleavage of neuropeptides and incretins on the stimulation of insulin secretion in β-cells. The truncated forms of GIP and GLP-1, and probably also of VIP and PACAP have no insulin-stimulating activity. NPY loses its capacity to stimulate the pancreatic Y₁ receptor after cleavage by DPP-IV, but the effect on insulin secretion is unknown. The truncated form of GRP retains its receptor-stimulating activity. PS, parasympathetic neuron; OS, orthosympathetic neuron. (—) loss of receptor signalling after truncation by DPP-IV. (Reproduced from Critical Reviews in Clinical Laboratory Sciences 2003; 40(3):209-94.)

A.3.3 Rationale for use of DPP-IV inhibition in type 2 diabetes

The main rationale for using DPP-IV inhibition in the treatment of type 2 diabetes is the prolongation of action of the incretin hormone, GLP-1 that has antidiabetic properties [7]. Moreover, patients with type 2 diabetes have reduced circulating concentrations of active GLP-1 after meal ingestion [76]. Therefore, inhibition of DPP-IV prevents inactivation of GLP-1 and tends to normalise GLP-1 levels in type 2 diabetes. As a result, the long-term actions of the hormone on β-cell neogenesis and apoptosis may be instituted [77], which may lead to a disease modification by preserving long-term β-cell function in patients with type 2 diabetes.
A.3.4 Other functions of DPP-IV

Wide range of functions has been attributed to DPP-IV. Some of these are mediated by the exopeptidase activity, like the processing of bioactive peptides and the involvement in the resorption of proline-containing peptides. The roles of DPP-IV in the immune system and tumour invasion appear to involve both enzymatic and non-enzymatic actions. Studies with DPP-IV-negative animals and in vivo inhibition experiments contribute to the insight into the physiological role of this protein. The role of DPP-IV/CD26 within the immune system appears to be a combination of its exopeptidase activity and its capacity to serve as a receptor or ligand for different molecules. This enables DPP-IV/CD26 to serve as a co-stimulatory surface molecule, to influence T-cell activity, and to modulate chemotaxis.

A.3.5 Tolerability of DPP-IV inhibition

The clinical studies conducted have shown that DPP-IV inhibition in human is highly tolerable and safe []. In the first 52-week efficacy and tolerability study of a DPP-IV inhibitor, LAF 237 appears to be well tolerated [78]. A major concern with DPP-IV inhibitors is the involvement of DPP-IV enzyme in the inactivation of biopeptides involved in the immune system[]. Although detailed studies on the immune system during treatment with DPP-IV inhibition has not yet been undertaken, there is no indication of altered immune function during the treatment[]. No evidence of effects of adverse events mediated by DPP-IV inhibition has been seen in clinical trials though DPP-IV inactivates various neuropeptides. The lack of such effect might be explained by the fact that most of these bioactive peptides have other inactivation systems apart from DPP-IV and, therefore, DPP-IV inhibition will not cause a complete prevention of their inactivation. Also, the clinically used DPP-IV inhibition has not been found to be 100% throughout the day for a long period of time. Elucidation of these mechanisms is beyond the scope of this thesis work.

In the present work, an attempt is made in exploring the utility of GRC 8011, a DPP-IV inhibitor, in the therapeutic management of type 2 diabetes. Broad outline of the study scheme is presented in Figure A.18.
STUDY SCHEME

Test compound

\[ \text{In vitro screening} \]

\[ \text{Acute toxicity} \quad \text{Preliminary Pharmacokinetics} \]

\[ \text{Preliminary in vivo screening} \quad \text{OGTT in mice} \]

\[ \text{Profilation experiments in different animal models} \]

\[ \text{Combination therapy in mice/rats} \quad \text{Repeated dose toxicity in rodent} \]

Figure A.18. Scheme of present study.