Chapter 1

Introduction

Prelude

A dramatic change in the lifestyle and dietary patterns has occurred worldwide during the last century across the populations. The marked variations in human environments and the accompanied globalization accelerated these changes. The switching of work culture from heavy labour to sedentary, increased mechanization, improved transportation facilities are some of the changes that have had an impact on human health. Physical inactivity and excessive intake of energy-dense and nutrient-poor food options are the two major behavioural contributors to the incidence and prevalence of non-communicable diseases (NCDs). NCDs such as diabetes and cardiovascular diseases have now become the main public health challenges for the 21st century and affect the productivity of people. In both developed and developing nations, diabetes epidemic, particularly type 2 diabetes (T2DM) remains as a major threat. Paradoxically, this also relates to the achievements in public health care during the 20th century that resulted in the increase of average life expectancy owing to the elimination of many of the communicable diseases. In the absence of effective and affordable interventions for both types of diabetes, the frequency of diabetes will be further escalating worldwide. Even though, researchers and clinicians are involved in the study and development of various pharmacological interventions, diabetes and its secondary complications continue to be a global epidemic and there is high demand for novel treatment options without much adverse effects. The increasing prevalence of diabetes and obesity has already imposed a huge burden on health-care systems and this will continue to increase in the future. Although T2DM is numerically more prevalent in the general population and type 1 diabetes is restricted to a small population of children; the condition may be reversed within one to two decades because of the increasing prevalence of T2DM in children and adolescents.
1. 1 Diabetes

“Diabetes is a mysterious illness”, the statement by Greek physician Araetaus around 130 AD is still relevant\textsuperscript{11}. Diabetes mellitus is a major epidemic of this century\textsuperscript{12} which has increased in incidence by 50\% over the past 15 years\textsuperscript{13}. Despite, this mysterious and multifactorial illness has become the modern epidemic; the surprising fact is that, diabetes is one of the world’s oldest diseases, described in historical records of civilizations such as those found in ancient Egypt, Persia, and India\textsuperscript{14-16}. Diabetes mellitus is a heterogeneous disorder characterized by chronic hyperglycaemia and the etiological heterogeneity is suggested by genetic inheritance and its interplay with environmental factors\textsuperscript{17}. One theory concerning the etiology of diabetes tells that it is the result of the evolution of a thrifty genotype that had survival benefits in the past but is detrimental in the current environment; the opposing theory is that diabetes represents an adult metabolic response to fetal malnutrition\textsuperscript{18}.

1. 2 History of diabetes

The first reference to diabetes mellitus in the history was described about 3500 years ago by the ancient Egyptians in ‘Ebers Papyrus’ (1550 BC) that mentions remedies for the treatment of excessive urination or polyurea and has detected by German Egyptologist Georg Ebers in 1872 from Thebes\textsuperscript{19, 20}. Initially, the term ‘diabetes’ was introduced by Araeteus of Cappodocia (81-133 AD) from the Greek word for siphon\textsuperscript{14}. The Greek medical writer Galen, a contemporary of Aretaeus mentioned the condition as “diarrhea of the urine” and “the thirsty disease.” Indian physicians Charaka and Sushruta noted the attraction of flies and ants to the urine of those affected by this ailment, coined the term ‘madhumeha’ or ‘honey urine’ and also detected that patients suffering from madhumeha exhibited extreme thirst and foul breath; a thousand years before the first Europeans recognized the sweet taste of urine in patients with diabetes\textsuperscript{19}. The word ‘mellitus’ (honey sweet) was added by Thomas Willis from Britain in 1675 after rediscovering the sweetness of urine and blood of patients\textsuperscript{14}. In 1776, British researcher Matthew Dobson gave first experimental evidence for the excretion of sugar in the urine of people suffering from diabetes in a paper presented to the medical society of London\textsuperscript{19}. In 1857, Claude Bernard from France discovered the role of liver in diabetes and it was established
through the identification of the fact that glycogen is stored in liver as the precursor of glucose\textsuperscript{14}.

1.2.1 Discovery of insulin

The key milestone in the history of diabetes is the discovery of role of the pancreas in pathogenesis of diabetes by Austrian researchers Mering and Minkowski in 1889\textsuperscript{21}. Minkowski hypothesised that pancreas produces a substance which regulate blood glucose level. In 1893, French scientist Gustave-Edouard Laguesse suggested that tiny islands of pancreatic tissue described by Paul Langerhans in 1869 might be the source of the substance involved in blood glucose control. Distinguished German pathologist, Paul Langerhans, a student of Rudolf Virchow described small groupings of pancreatic cells that were not drained by pancreatic ducts in his doctoral thesis. Belgian physician Jean de Mayer named the presumed substance produced by the islets of Langerhans “insulin” in 1909\textsuperscript{22}. Moses Barron reported a rare case of a pancreatic stone in 1920 that blocked the main pancreatic duct and the blockage caused the degeneration of the acinar glandular cells but not the islet cells. This report by Barron as well as the hypothesis put forward by Minkowski stimulated research ideas of Frederick Grant Banting, an orthopedic surgeon who joined in John J. R Macleod’s laboratory at University of Toronto, Canada. The idea was to surgically ligate the pancreas to stop the flow of nutrients towards acinar glandular cells, which may cause the disintegration of these exocrine cells that produces digestive pancreatic juice and preservation of the islet cells for the preparation of pancreatic islet extracts\textsuperscript{23}. In 1921, Banting along with the physiology student Charles Best and biochemist James Collip succeeded in purifying an active extract from atrophied pancreatic glands of the laboratory dogs and was effectively reversed the diabetic symptoms in pancreatectomized diabetic dogs\textsuperscript{24}. Banting named the isolated antidiabetic substance as ‘isletin’ but Macleod suggested the name ‘insulin’ without knowing that the name ‘insuline’ had already been coined by de Mayer in 1909\textsuperscript{23}. Till the early 20\textsuperscript{th} century the life expectancy of children with type 1 diabetes was only about 2 years. The news of the discovery of insulin was accepted internationally with tremendous enthusiasm and thus many children suffering from diabetes were reinstated to health. In 1923, Banting and Macleod were awarded Nobel Prize for the discovery of insulin.
1. 3 Definition and classification of diabetes

According to World Health Organization (WHO) diabetes is defined as group of metabolic disorders characterized by hyperglycemia with disturbances in carbohydrate, protein and lipid metabolism resulting from defects in insulin secretion, insulin action or both\(^{25}\). Diabetes can be raised from a variety of abnormalities. These ranges from mechanisms that cause pancreatic beta-cell degeneration or cessation of insulin production and secretion to conditions that cause insulin insensitivity to peripheral tissues\(^{26}\). The insensitivity or lack of insulin results in deficient insulin action on its target organs and results in deranged carbohydrate, protein and lipid metabolism\(^{27}\). Based on the pathogenesis of hyperglycemia diabetes is classified into 3 etiological types along with some minority cases due to various specific metabolic or genetic causes\(^{28}\). The various forms of diabetes is listed in Table 1. 1.

<table>
<thead>
<tr>
<th>Classification of Diabetes Mellitus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1 diabetes mellitus</td>
</tr>
<tr>
<td>Type 2 diabetes mellitus</td>
</tr>
<tr>
<td>Gestational diabetes</td>
</tr>
</tbody>
</table>

**Other forms:**

<table>
<thead>
<tr>
<th>Pancreatic disease:</th>
<th>chronic pancreatitis, pancreatectomy, cystic fibrosis, haemochromatosis, pancreatic carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endocrine:</td>
<td>acromegaly, Cushing’s syndrome, thyrotoxicosis, phaeochromocytoma</td>
</tr>
<tr>
<td>Drug induced:</td>
<td>corticosteroids, thiazide diuretics</td>
</tr>
<tr>
<td>Conditions with specific genetic causes:</td>
<td>e.g., maturity-onset diabetes of the young (MODY)</td>
</tr>
</tbody>
</table>

Table 1. 1. Classification of diabetes mellitus (reproduced from the reference 28 with permission).

1. 3. 1 Type 1 Diabetes

Type 1 diabetes (T1DM) was previously termed as insulin dependent diabetes mellitus (IDDM) or juvenile onset diabetes. T1DM accounts for about 5-10% of diabetes prevalence results from a cellular mediated autoimmune destruction of insulin producing pancreatic beta cells, leading to absolute insulin deficiency\(^{26}\). Patients with T1DM has been found to carry a number of autoantibodies in their blood targeted to pancreas, the
site of production and secretion of insulin such as islet cell antibodies (ICA), insulin autoantibodies (IAA), and antibodies against glutamic acid decarboxylase (GAD) and insulinoma-associated autoantigen-2 (IA-2), a proteins found in pancreatic beta-cells. Development of chronic hyperglycemia and keto-acidosis are the major manifestations of IDDM. The rate of pancreatic beta cell degeneration varies from individual to individual under this condition which may be rapid in infants and children; this can be reason behind the early classifications like juvenile onset diabetes.

1.3.2 Type 2 Diabetes

Type 2 diabetes is the global epidemic that constitutes about 90-95% of those with diabetes and referred to as non-insulin dependent diabetes (NIDDM) or adult onset diabetes. The advancement of the etiology of T2DM results from the combination of impaired biological response to insulin i.e insulin resistance and relative insulin deficiency and the subsequent derangement in carbohydrate, protein and lipid metabolism. The progression from normal glucose tolerance to abnormal glycemia results from these two fundamental defects in the pathogenesis of T2DM and is caused by the combination of genetic and environmental factors (Figure 1.1). The genetic factors may be primary or secondary; primary genetic factors include the genes responsible for diabetes or ‘diabetogenes’ and the diabetes induced modulations in gene expression constitute secondary factors. Environmental factors such as diet, obesity and physical activity may act as initiating factors or progression factors for T2DM.

1.3.3 Gestational Diabetes

Any degree of glucose intolerance developed during the pregnancy period is termed as gestational diabetes mellitus (GDM). In the early phase of pregnancy i.e first trimester and first half of second trimester, the fasting and postprandial glucose concentrations are normally lower than in normal, non-pregnant women. So elevated fasting or postprandial plasma glucose levels at this time in pregnancy may well reflect the presence of diabetes. One of the undesirable effects of GDM is that there are chances to continue to be in hyperglycaemic state by the person with GDM even after the delivery and further progression to T2DM.
1. 4 Diabetes prevalence - Global burden and ethnicity

The number of people living with diabetes and mortality due to diabetes across the world is shocking\textsuperscript{31}. Currently there are 387 million people with diabetes worldwide whereas 175 million people with diabetes are undiagnosed and is expected to affect 592 million people by 2035\textsuperscript{13}. Diabetes caused 4.9 million deaths in 2013 and in every 7 seconds a person dies from diabetes\textsuperscript{13}. The incidence (number of new cases per year per unit of population) and prevalence (number of known cases per unit of population) of diabetes vary significantly with geographical locations\textsuperscript{28}. Europe has the highest prevalence of T1DM in children and lowest prevalence in Japan\textsuperscript{13}. T2DM has the highest prevalence in Pima Indians, Pacific Islanders, South Asians, Hispanics, and Africans\textsuperscript{28}. South Asian region which constitute India and China forms one of the epicenters of the global diabetes epidemic\textsuperscript{31,32}. Investigations in the South Asian population residing in the UK during the early 1980s suggested the possibility of an Asian Indian or South Asian phenotype with a characteristic metabolic profile as shown in Figure 1. 2. South Asian phenotype refers to a combination of characteristics that predisposes South Asian to the development of insulin resistance, T2DM, and cardiovascular disease\textsuperscript{32}. T2DM occurs at younger ages and at
lower levels of BMI in South Asian compared with Caucasians\textsuperscript{33}. In spite of a relatively lower rate of obesity as defined by BMI cut points, South Asian tend to have larger waist measurements and waist-to-hip ratios, indicating a greater degree of central body obesity\textsuperscript{34}. In India, 65 million people are diabetic, the nationwide prevalence is around 9\%, but in the relatively prosperous southern cities it is as high as 20% \textsuperscript{31}. Surprisingly, Kerala the most literate and socially developed state of India is reported to have 27\% of diabetes population even with the availability of high class medical facilities\textsuperscript{35}. This is a very alarming situation and need detailed investigation and intervention to curtail the uncontrolled growth of this metabolic disorder. The study by Bakker et al. suggests that only a short term high fat, high calorie diet impairs insulin sensitivity in healthy, young South Asian men but not in Caucasian men\textsuperscript{36}. Thus, Indians carrying the South Asian phenotype and having a tendency to follow a sedentary lifestyle along with a high fat, high carbohydrate, and high calorie dietary pattern suggest the possible means of epidemic of T2DM in this subcontinent.

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{figure1.png}
\caption{Metabolic profile of South Asian (Asian Indian) phenotype (reproduced from the reference 32 with permission).}
\end{figure}

\section{5 Diagnosis}

The established glucose criteria for the diagnosis of diabetes include the fasting plasma glucose (FPG) and 2 hour postprandial glucose (2 h PG) during an oral glucose tolerance test (OGTT). The prescribed diagnostic cut point for FPG is \textit{\textgreater}126 mg/dl (7.0 mM) and
that of diagnostic 2-h PG value is $\geq 200$ mg/dl (11.1 mM)$^{26}$. According to WHO, OGTT should be performed using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water to find out the 2-h PG value$^2$. A random (or casual) plasma glucose analysis is also required for patients with severe classic hyperglycemic symptoms or hyperglycaemic crisis$^{26}$. The American Diabetes Association (ADA) diagnostic criteria for diabetes are given in Table 1. For the initial assessment of the severity of the diabetes an HbA1c test is also recommended by ADA as HbA1c is a widely used marker of chronic glycemia, reflecting average blood glucose levels over a 2 to 3 months period of time. Diagnostic cut point of HbA1c is 6.5% and this test correlates well with microvascular and, to a lesser extent, macrovascular complications and is widely used as the standard biomarker for the adequacy of glycemic management$^{37}$. The HbA1c test has several advantages over the FPG such as greater convenience, fasting is not required, greater pre-analytical stability and less day-to-day perturbations during periods of stress and illness$^{26}$.

<table>
<thead>
<tr>
<th>Blood glucose parameter</th>
<th>% Glycated Hemoglobin (% HbA1c)</th>
<th>Fasting plasma glucose (FPG)</th>
<th>Postprandial plasma glucose (PPG)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>$&lt; 6.5$</td>
<td>$&lt; 100$ mg/dL</td>
<td>$&lt; 140$ mg/dL</td>
</tr>
<tr>
<td>Prediabetes</td>
<td>6.5 - 7</td>
<td>100 - 125 mg/dL</td>
<td>140 - 199 mg/dL</td>
</tr>
<tr>
<td>Diabetes</td>
<td>$&gt; 7$</td>
<td>$&gt; 126$ mg/dL</td>
<td>$&gt; 199$ mg/dL</td>
</tr>
</tbody>
</table>

Table 1. Criteria for the diagnosis of diabetes (reproduced from ADA guidelines, 2011 with permission)$^{141}$

*2-h plasma glucose $> 199$ mg/dl (11.1 mmol/l) during an OGTT. The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.

OR

In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose $> 199$ mg/dl (11.1 mmol/l).

1. 5. 1 Prediabetes

Prediabetes is a condition where blood sugar level is higher than normal, but not enough to be classified as diabetes. The term impaired glucose tolerance (IGT) or prediabetes was first coined in 1979 by the World Health Organization and the National Diabetes Data Group to replace the terms *borderline* and *asymptomatic diabetes mellitus*. 

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*141*
In 1997, an expert committee of the American Diabetes Association recommended the following criteria for IGT: a normal fasting plasma glucose (<126 mg/dL) with a postprandial plasma glucose of >140 mg/dL but <200 mg/dL 2 h after a 75 g oral glucose challenge. The pre-diabetic stage is an extremely useful marker of patients at risk for the eventual development of type 2 diabetes. Patients with IGT may benefit from timely patient education and perhaps even more aggressive forms of intervention or medication. Prediabetic stage is the warning sign for diabetes; subjects in the prediabetic stage may be return to normal stage with careful diet and exercise.

1. 6 Insulin

Insulin is a peptide hormone secreted by the beta-cells of the pancreatic islets of Langerhans and maintains normal blood glucose levels by facilitating cellular glucose uptake, regulating carbohydrate, lipid and protein metabolism and promoting cell division and growth through its mitogenic effects. Insulin is the dipeptide containing A chain and B chain linked by disulphide bridges, and composed of 51 amino acids with a molecular weight of 5802. The A chain comprises 21 amino acids and the B chain 30 amino acids (Figure 1.3A). Its iso-electric point is pH 5.5. In 1958, Frederick Sanger was awarded his first Nobel Prize for determining the sequence of the amino acids that make up insulin.

Insulin is coded on the short arm of chromosome 11 and synthesised as its precursor, proinsulin. Initially, Pre-proinsulin is formed by sequential synthesis of a signal peptide, the B chain, the connecting (C) peptide and then the A chain comprising a single chain of 100 amino acids in the ribosomes of the rough endoplasmic reticulum (RER) from the mRNA. Removal of the signal peptide gives rise to proinsulin, which acquires its characteristic 3 dimensional structure in the endoplasmic reticulum. Proinsulin is transferred from the RER to the Golgi apparatus through secretory vesicles; aqueous zinc and calcium rich environment in the Golgi favours formation of soluble zinc-containing proinsulin hexamers. The immature storage vesicles are formed from the Golgi and enzymes acting outside the Golgi convert proinsulin to insulin and C peptide. Insulin forms zinc-containing hexamers which are insoluble, precipitating as chemically stable crystals at pH 5.5. When mature granules are secreted into the circulation by exocytosis,
insulin, and an equimolar ratio of C-peptide are also released. Proinsulin and zinc typically comprise no more than 6% of the islet cell secretion\textsuperscript{43}.

1. 6. 1 Insulin secretion and factors involved

Elevated blood glucose level induces the ‘first phase’ of glucose stimulated insulin secretion (GSIS) by release of insulin from secretory granules of the beta-cell. Glucose entry into the beta cell is sensed by glucokinase, which phosphorylates glucose to glucose-6-phosphate (G6P) which increases rate of glycolysis and ATP production\textsuperscript{45}. High intracellular ATP level cause the closure of K\textsuperscript{+}-ATP-dependent channels which results in membrane depolarization and activation of voltage dependent calcium channels leading to an increase in intracellular calcium concentration; this triggers pulsatile insulin secretion\textsuperscript{46}. Other mediators of insulin release include activation of phospholipases and protein kinase C (for example by acetylcholine) and by stimulation of adenylyl cyclase activity and activation of beta cell protein kinase A, which potentiates insulin secretion. This adenylyl cyclase mediated mechanism may be activated by hormones, such as vasoactive intestinal peptide (VIP), pituitary adenylate cyclase-activating polypeptide, glucose-dependent insulino tropic polypeptide (GIP) and glucagon-like peptide-1(GLP-1) (Figure 1. 3B). These factors appear to play a significant role in the second phase of glucose stimulated insulin secretion, after refilling of secretory granules translocated from reserve pools\textsuperscript{47}.

It was found that insulin secretion from the islet cells into the portal veins is characteristically pulsatile, by the summation of coordinate secretory bursts from millions of islet cells\textsuperscript{40}. Reports also suggest that insulin is more effective at reducing blood glucose levels if it is delivered in pulses rather than continuously\textsuperscript{48}. In healthy individuals glucose stimulated insulin secretion from pancreas is biphasic: an initial component (first phase), which develops rapidly but lasts only a few minutes, followed by a sustained component (second phase)\textsuperscript{49, 50}. The first phase starts with a rapid rise in insulin 1-3 minutes after the plasma glucose elevation and it returns towards baseline 6-10 minutes after glucose stimulation; in the second phase the insulin level raises gradually once again\textsuperscript{51}. Loss or diminished first-phase secretion and reduced second-phase secretion are characteristic features of T2DM and it is well known that a decrease in the first phase of
GSIS is found in the early stage of T2DM (Figure 1.4) and also in impaired glucose tolerance (IGT) \textsuperscript{49, 52}. The first phase of insulin secretion represents release of insulin already synthesised and stored in secretory granules; the second phase represents secretion of both stored and newly synthesised insulin.

\textbf{Figure 1.3.} (A) Formation of insulin from proinsulin. (B) Schematic representation of factors involved in the regulation of insulin secretion (reproduced from the reference 40 with permission).

Synthesis and secretion of insulin is regulated by nutrient and non-nutrient secretagogues, in addition to environmental stimuli and the interactions of other hormones\textsuperscript{44}. Nutrient secretagogues such as glucose appear to trigger insulin secretion from the beta-cell by increasing intracellular ATP and closing of K\textsuperscript{+}-ATP channels. Other factors involved are amino acids, fatty acids, pituitary adenylate cyclase-activating polypeptide, GIP, GLP-1,
acetylcholine, adipokines like leptin and adiponectin. Non-nutrient secretagogues may act via cholinergic and adrenergic pathways, or through peptide hormones and cationic amino acids. When food is seen, smelled or acutely ingested, islet cell cholinergic muscarinic receptors activate phospholipase C, with subsequent intracellular events activating protein kinase C, phospholipase A2 and mobilizing intracellular calcium and promote insulin secretion. Catecholamines, through alpha 2-adrenoceptors, typically inhibit insulin release during stress and exercise. Nutrients in the GI tract stimulate the secretion of hormones known as incretins which amplify glucose-induced insulin release. These account for the greater insulin response to oral, as opposed to intravenous, glucose.

Figure 1.4. Dynamics of insulin secretion in obesity, IGT, and T2DM (reproduced from the reference 226 with permission) (A) In obesity, the function of individual beta-cells appears to be normal and both phases of GSIS are enhanced primarily due to an increase in beta-cells. (B) In IGT, the first phase is slightly impaired because of a decrease in the size of the readily releasable pool (RRP) and/or partial defect in the exocytotic process of the granules in this pool, and the second phase is only moderately reduced. (C) In T2DM, the first phase is absent because of a complete loss of the RRP and/or a complete defect in the exocytotic process. The second phase is also reduced, probably due to the decreased releasable pool (RP) and/or disturbance of cortical actin network in T2DM. Black lines indicate dynamics of insulin secretion in normal state, red lines indicate insulin secretion under T2DM.

GIP and GLP-1 are the two most important incretin hormones. GLP-1 enhances insulin action by cAMP generation and activation of a cAMP-responsive protein kinase. Adiponectin appear to act via glucose phosphorylation, calcium influx and protein kinase C. Marked variation in insulin secretion may occur via modulation on beta cell mass and differentiation.
1. 6. 2 Physiological actions of insulin

Insulin is a key player in the regulation of intermediary metabolism and it organizes the use of fuels for either storage or oxidation. By controlling these processes, insulin has profound influences on both carbohydrate and lipid metabolism, and significant effects on protein and mineral metabolism. Hence, derangements in insulin signalling either due to insulin deficiency or insulin resistance have widespread and devastating effects on many organs and tissues.

1. 6. 3 Effect of insulin in glucose metabolism

Insulin has multiple actions on different tissues of the body and is the major regulator of blood glucose concentrations. As a result of overnight fasting, insulin levels remains low and blood glucose concentrations are maintained by the hepatic glucose supply. Under the fed state, the intestine digests the food and the glucose produced is absorbed from the gut, causing an increase in blood glucose concentration. The rise in blood glucose concentration stimulates the release of insulin by pancreatic beta-cells. Insulin reduces blood glucose mainly in two ways; by reducing the supply of glucose from the liver as well as by increasing the uptake of glucose by organs such as muscle and fat tissue. The concentration of insulin required to reduce glucose supply by the liver is probably lower than that needed to increase the uptake of glucose by muscle and fat28.

1. 6. 4 Effect of insulin in protein metabolism

Insulin has an anabolic effect in protein metabolism in which it promotes amino acid uptake by cells and conversion of these amino acids into protein56, 57. In vivo studies report that the main effect of insulin is the reduction of proteolysis and maintenance of protein turnover in muscle and liver rather than a stimulation of protein synthesis58, 59. Negative nitrogen balance related to weight loss is one of the symptoms of diabetes which is promoted by the lack of insulin action on protein degradation under insulin deficient states.
1. 6. 5 Effect of insulin in lipid metabolism

Major effect of insulin in fat metabolism is the reduction in the plasma free fatty acids (FFA) level. It inhibits hormone-sensitive lipase (HSL) which catalyses breakdown of triglycerides (lipolysis) in adipose tissue; thus decreasing the release of FFA from adipocytes. FFA released from adipose tissue may be used by skeletal muscle and liver as a fuel source during fasting or under exercise, thus reserving the supply of glucose as a fuel for other tissues such as brain. Increased circulating insulin concentration inhibits hepatic VLDL secretion, largely via suppression of FFA availability. Insulin promotes *de novo* lipogenesis by the induction of several key enzymes of lipogenic pathway, including fatty acid synthase and acetyl-CoA carboxylase and it also reduces the level of circulating ketone bodies.

1. 6. 6 Molecular mechanism of insulin signalling

Insulin exerts its metabolic and mitogenic effects on target organs such as liver, muscle, and fat through its cell surface receptors and by initiating a number of intracellular signalling cascades. Insulin receptors (IR) are located on the plasma membrane of the insulin-sensitive tissues and the hormone binds reversibly to these cell surface transmembrane receptors. When insulin binds to its receptor, it induces a conformational change in the insulin receptor molecule; this increases its tyrosine kinase activity, which then autophosphorylates multiple tyrosine residues within its intracellular region and insulin receptor substrates (IRS). The phosphorylated tyrosine residues on the insulin receptor create binding sites for a number of soluble intracellular effector proteins that attach to the insulin receptor and are phosphorylated by the insulin receptor tyrosine kinase. The effector proteins/enzymes activated by phosphorylation causes the activation of a number of divergent intracellular signalling cascades involving other tyrosine kinases, serine kinases, and lipid kinases. The key enzymes participating in this intracellular signalling are lipid kinase PI 3-kinase, protein kinase B or AKT, glycogen synthase kinase-3, and certain isoforms of protein kinase C. Schematic representation of insulin signalling pathways is given in Figure 1.5.
The major metabolic effects of insulin are acceleration of glucose uptake into skeletal muscle and adipose tissue, and stimulation of glycogen storage in the liver. Insulin promotes the translocation of the glucose transporter GLUT-4 from the intracellular storage vesicles to the cell membrane surface in insulin-sensitive tissues, allowing glucose to enter cells down its concentration gradient. This insulin action on cellular glucose uptake depends on the upstream signalling molecules PI 3-kinase and protein kinase C. Glucose transporters are the members of major facilitator superfamily and they mediate the thermodynamically downhill movement of glucose across the plasma membrane of animal cells. A brief description of major mammalian glucose transporters are given in Table 1. The effect of insulin on glycogen synthesis is partly due to PI 3-kinase-dependent inhibition of glycogen synthase kinase-3.

1.7 Physiology and biochemistry of glucose homeostasis

Glucose is the essential nutrient and acts as the starting material for most of the biosynthetic pathways in the human body. Glucose is the major fuel for the tissues. Glucose is the exclusive energy source for brain and red blood cells under non starvation conditions, which depend on the bloodstream for a steady glucose supply. The liver plays a central role in the glucose homeostasis by balancing the uptake and storage of glucose via glycogenesis and the release of glucose via glycolysis and gluconeogenesis. Once the liver glycogen store is full, the adipose tissue converts glucose into triacylglycerol for longer term storage as fat. Muscles utilises the major share of plasma glucose and it also efficiently accumulate glycogen for their own use. The brain is a

**Figure 1.5.** Schematic presentation of insulin signalling pathways (reproduced from the reference 227 with permission).
particular target organ that can use glucose and/or ketone bodies as an energy source. However, the fact that glucose represents the sole source of energy for some of its cells imposes a tight control over glycemia\textsuperscript{71}.

<table>
<thead>
<tr>
<th>ISOFORM</th>
<th>Tissue Distribution</th>
<th>Affinity for Glucose</th>
<th>Km</th>
<th>Characteristics</th>
<th>Gene location</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLUT 1</td>
<td>Brain micro vessels, Red blood cells Placenta Kidney All tissues</td>
<td>High</td>
<td>1 mmol/L</td>
<td>Ubiquitous Basal transporter</td>
<td>Chr 1</td>
</tr>
<tr>
<td>GLUT 2</td>
<td>Liver Kidney beta cell Small intestine</td>
<td>Low</td>
<td>15-20 mmol/L High Km</td>
<td>Insulin-independent transporter</td>
<td>Chr 3</td>
</tr>
<tr>
<td>GLUT 3</td>
<td>Brain neurons Placenta Foetal muscle All tissues</td>
<td>High</td>
<td>&lt;1 mmol/L Low Km</td>
<td>Found in glucose dependent tissues</td>
<td>Chr 12</td>
</tr>
<tr>
<td>GLUT 4</td>
<td>Muscle cells Adipocytes Heart</td>
<td>Medium</td>
<td>2.5-5 mmol/L</td>
<td>Sequestered intracellularly and translocates to cell surface in response to insulin</td>
<td>Chr 17</td>
</tr>
<tr>
<td>GLUT 5</td>
<td>Small intestine Testes</td>
<td>Medium</td>
<td>6 mmol/L</td>
<td>High affinity for fructose</td>
<td>Chr 1</td>
</tr>
</tbody>
</table>

Table 1. 3. A brief description of major mammalian glucose transporters (reproduced from the reference 69 with permission).

1. 7. 1 Liver - the major metabolic regulatory organ

Liver is the key regulator of glucose metabolism and the absorbed glucose reach the liver before being delivered to muscle and adipose tissue. These are the notable factors that make liver as unique organ in carbohydrate homeostasis\textsuperscript{72}. The glucose-rich blood from the digestive tract directly reaches to liver through the portal vein. The liver cells have a large number of GLUT-2 transporters for the intracellular glucose transport whose function is independent of insulin action. GLUT-2 has a high Km value or low affinity for
glucose which enables a rapid influx of glucose when plasma glucose levels are high\(^69\). Hence, levels of glucose inside and outside the cell can become equal in liver. The liver possesses a unique enzyme glucokinase (GK) for the conversion of glucose into glucose 6 phosphate (G6P) to trap inside the cell, rather than hexokinase, the enzyme present in other tissues. The peculiarity of GK is that it can produce G6P at a faster rate and is not inhibited by its product\(^69\). Thus, liver can work as major consumer of glucose and it buffers plasma glucose level through glycolysis, glycogenesis and lipogenesis under the influence of insulin\(^70\). Once trapped inside the liver cell, under the fed state, the high levels of insulin and low levels of glucagon stimulate glycolysis, which can be non-oxidative type, producing pyruvate or oxidative type, by releasing acetyl CoA which is further oxidized through the tricarboxylic acid (TCA) cycle to form ATP, carbon dioxide and water\(^69\). The generated ATP is used for anabolic and other energy-requiring processes in the cell. Non-oxidative glycolysis carbons undergo gluconeogenesis and the newly formed glucose is either stored as glycogen or released back into plasma. Elevated glucose and insulin both stimulate metabolic enzymes involved in glycogen synthesis and this accelerates further hepatic glucose uptake and storage as glycogen. Glycogenesis is promoted by insulin through the activation of glycogen synthase and inhibition of glycogen phosphorylase\(^69\).

When the liver glycogen stores get replenished, the excess glucose in the liver is converted to triacylglycerols (TG). TG possesses two components; the glycerol and the fatty acid moieties and both can be synthesized from glucose. Under normal physiological conditions, liver does not store triacylglycerols, instead the TG is packaged along with proteins, phospholipids, and cholesterol into the lipoprotein complexes known as very low-density lipoproteins (VLDL), which are secreted into the bloodstream. Some of the fatty acids from the VLDL are taken up by tissues for their immediate energy needs, but most are stored in adipose tissue as triacylglycerols\(^73\).

When the plasma glucose level falls, the liver has a critical role in regulating endogenous glucose production from de novo synthesis (gluconeogenesis) or the catabolism of glycogen (glycogenolysis)\(^74\). The previous studies suggest that approximately 75\% of hepatic glucose release is derived from glycogenolysis, and the remainder (25\%) from gluconeogenesis, primarily by the conversion of lactate and alanine to glucose\(^75, 76\). The
Figure 1. 6 shows the anabolic and catabolic pathways occurring in the liver.

**Figure 1. 6.** Anabolic and catabolic reactions occurring in liver. Glucose metabolism involves both energy producing (catabolic) and energy consuming (anabolic) reactions. (reproduced from the reference 228 with permission).

### 1.8 Pathophysiology of diabetes

The core patho-physiologic defects in T2DM are insulin resistance in peripheral tissues like liver, adipose and muscle as well as beta cell failure. It is now recognized that the beta-cell failure occurs much earlier and is more severe than previously thought. Individuals with an elevated impaired glucose tolerance (IGT) are insulin resistant and have lost over 80% of their beta-cell function. In addition to the insulin resistance in muscle, liver, and beta-cell, accelerated lipolysis in adipose, incretin deficiency/resistance in the gastrointestinal tract, hyperglucagonemia in beta-cell, increased glucose reabsorption by the kidney, and insulin resistance in brain all play important roles in the development of glucose intolerance in type 2 diabetic individuals. The epidemic of diabetes that has enveloped westernized countries is related to the epidemic of obesity and physical inactivity. The metabolic staging of diabetes is illustrated in Figure 1. 7.
Insulin resistance is defined as reduced responsiveness towards normal circulating concentrations of insulin exhibited by the insulin dependent peripheral tissues such as liver, muscle and adipose towards insulin action\textsuperscript{63, 79, 80}. Genetic predisposition is a major factor in the origin and development of the insulin resistance\textsuperscript{81, 82}.

In liver, the insulin resistance is manifested by the overproduction of glucose during the basal state despite the presence of fasting hyperinsulinemia and an impaired suppression of hepatic glucose production in response to insulin after food intake\textsuperscript{82-84}. The insulin resistance in the muscle is developed by impaired glucose uptake following ingestion of a carbohydrate meal and results in postprandial hyperglycemia\textsuperscript{85-88}. Inflammation has an important role in the pathogenesis of insulin resistance, especially in adipocytes\textsuperscript{89}. Nowadays, it is clear that obesity is associated with a state of chronic, low-grade inflammation, particularly in white adipose tissue\textsuperscript{90}. The proinflammatory cytokines or adipokines like TNF alpha, MCP-1, IL-6, leptin, resistin, visfatin and PAI-1 are able to induce insulin resistance\textsuperscript{89, 91}. The primary mechanism through which inflammatory signalling leads to insulin resistance is the inhibition of insulin signalling downstream of the insulin receptor. Exposure of cells to TNF alpha or elevated levels of free fatty acids stimulates inhibitory phosphorylation of serine residues of IRS-1\textsuperscript{92-94}. This phosphorylation reduces both tyrosine phosphorylation of IRS-1 in response to insulin

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1_7}
\caption{Metabolic staging of diabetes (reproduced from the reference 63 with permission).}
\end{figure}

**1.8.1 Insulin resistance**
and the ability of IRS-1 to associate with the insulin receptor and thereby inhibits downstream signalling and insulin action \(^{94-96}\).

Excessive intracellular lipid accumulation in “ectopic sites,” such as liver and muscle is another proposed mechanism for insulin resistance; the increased skeletal muscle triglyceride concentrations and hepatic steatosis are typical concomitant abnormalities of insulin resistance states \(^{97}\). Metabolism of excessive fatty acid loads can result in the generation of bioactive lipid products such as diacyl glycerols (DAGs). This stimulates protein kinase C (PKC) activity, which leads to inhibitory serine phosphorylation of insulin signalling components causing insulin resistance \(^{97, 98}\). Similarly, saturated fatty acids can be converted to ceramides that inhibit AKT activity and decrease insulin sensitivity \(^{99}\).

1. 8. 2 Pancreatic beta-cell dysfunction

Insulin resistance causes an increased metabolic demand for insulin in several tissues that can result in the development of hyperglycemia. By increasing pancreatic beta-cell mass and insulin gene expression, beta-cells synthesize and secrete more insulin to compensate the increased demand. Thus, period of near-normal glycemia or IGT can exist in which pancreatic beta-cells compensate for insulin resistance by hypersecretion of insulin. But at a particular point, the beta-cell compensation is followed by beta-cell failure, in which the pancreas fails to secrete sufficient insulin. This is the time point where prediabetic state or IGT is converted to diabetic state. The link between insulin resistance, beta-cell dysfunction and T2DM and the time line of development of diabetic complications is demonstrated in Figure 1. 8A and B. Chronic hyperglycemia or glucotoxicity \(^{100}\), chronic dyslipidemia or lipotoxicity \(^{101}\), or the combination of both, glucolipotoxicity \(^{102}\) have been postulated to contribute to the worsening of beta-cell function over time, creating a vicious cycle by which metabolic abnormalities impair insulin secretion, which further aggravates metabolic perturbations \(^{103}\). Moreover, the antioxidant defence system of pancreatic beta cell is not strong enough to withstand the deleterious free radical generated by the glucolipotoxicity \(^{104}\).
1. 8. 3 Physiological starvation and ketoacidosis

T1DM and also the end stage T2DM can be often referred to as “starvation in the midst of plenty”\textsuperscript{105}. Under diabetes, the extracellular glucose levels may be extremely high, but the cells are unable to uptake and utilise the glucose. As in starvation, these individuals use non-glucose sources of energy, such as fatty acids and ketone bodies, in their peripheral tissues. An uncontrolled production of ketone bodies may occur; these ketone bodies are weak acids and it acidifies the blood\textsuperscript{106}. The result is the metabolic state of diabetic ketoacidosis (DKA). Hyperglycemia and ketoacidosis are the hallmarks of insulin deficient diabetes, either type 1 or type 2\textsuperscript{107}. Hypertriglyceridemia can also be noticed in DKA. In diabetic subjects, the enzyme lipoprotein lipase that normally degrades lipoproteins is inhibited by the low level of insulin and the high level of glucagon. So, the levels of VLDL and chylomicrons derived from the lipid components of the diet are high under DKA\textsuperscript{105}.

1. 9 Diabetes secondary complications

Secondary complications of diabetes occur in the majority of individuals with both type 1 and type 2 diabetes. Prolonged hyperglycemia increases the likelihood of developing secondary damage to numerous systems, and these complications represent a substantial cause of morbidity and mortality\textsuperscript{108}. The duration and degree of hyperglycaemia, the protracted increase in blood glucose levels beyond the usual fasting or postprandial ranges positively correlates the occurrence of secondary complications\textsuperscript{109}. The most common complications of diabetes are ‘microvascular disease’ (due to damage to small blood vessels) and ‘macrovascular disease’ (due to damage to the arteries) which share numerous mechanisms by which hyperglycaemia can disrupt cell and organ functions.

Microvascular complications include eye damage or ‘retinopathy’, brain damage, kidney dysfunction termed ‘nephropathy’ and neural damage or ‘neuropathy’ and the major macrovascular complications include accelerated cardiovascular disease resulting in myocardial infarction and cerebro-vascular disease manifesting as strokes\textsuperscript{110}. The organs that are susceptible to diabetic complications carries the cells that exhibit insulin independent glucose uptake and possess the glucose metabolizing enzyme aldose reductase; for example capillary endothelial cells in the retina, mesangial cells in the renal
glomerulus, and neurons and Schwann cells in peripheral nerves \cite{108, 111}. Hence, diabetes selectively damages cells, like endothelial cells and mesangial cells, whose glucose transport rate does not depend on insulin levels, leading to the accumulation of glucose inside the cell \cite{111}.

![Diagram](image)

**Figure 1.8.** (A) The link between insulin resistance, beta cell function and diabetes (B) time line of development of macro and microvascular complications. (reproduced from the references 38 and 39 with permission).

Hyperglycemia accelerates the development of vascular complications via several mechanisms: activation of the polyol and hexosamine pathways, activation of protein kinase C, increased oxidative stress, increased production of advanced glycation end-products (AGEs), increased synthesis of growth factors, cytokines and angiotensin II \cite{112}. These factors in turn, induce endothelial dysfunction and lead to the progressive development of micro- and macrovascular complications and multiorgan damage \cite{112}.
Reports suggest that increased oxidative stress, induced by several hyperglycemia activated pathways, is a key factor in the pathogenesis of endothelial dysfunction and vascular disease\textsuperscript{113}. A number of intervention studies in type 2 diabetic patients have shown that intensive treatment is required to reduce the long-term complications of the disease\textsuperscript{18}.

1. 10 Diabetes treatment options

The first step in the progression from normal glucose tolerance to T2DM is IGT\textsuperscript{81, 114}. Impaired fasting glucose (IFG) and IGT are recognized as prediabetic states by the ADA\textsuperscript{26}. The characteristic features of IGT or IFG are impaired beta-cell function and insulin resistance. Hence, the first line diabetic interventions must be followed to prevent/slow IGT progression to diabetes that can preserve or augment beta-cell function and can ameliorate insulin resistance\textsuperscript{115}. The treatment regimen for diabetes must be designed to achieve specific target goals which may depend on the age of the patient, the years of anticipated survival, other concomitant illnesses, and the patient’s willingness to comply with specific treatment regimens\textsuperscript{18}. Diet and exercise, insulin and oral therapies as well as traditional remedies are the available options for the control and management of diabetes. But the universally accepted fact is that effectiveness of the diabetes therapies are solely based on individualised care and management of glycemia.

1. 10. 1 Diet and exercise

Obesity and physical inactivity are major risk factors for type 2 diabetes\textsuperscript{116}. Obesity is the single most important factor responsible for the marked increase in both the incidence and prevalence of T2DM over the last 20 years due to the pronounced changes in the human environment, behaviour and lifestyle\textsuperscript{1}. These have resulted in escalating rates of both obesity and diabetes and the recent adoption of the term ‘diabesity’, first suggested by Shafrir a decade ago\textsuperscript{117, 118}. Insulin resistance the key etiological factor of T2DM can be resulted from weight gain and physical inactivity. Obese individuals with a predominance of intra-abdominal or mesenteric fat have higher rates of FFA mobilization and greater resistance to the anti-lipolytic effects of insulin when compared with individuals with lower body obesity\textsuperscript{119, 120}. In contrast to this, insulin sensitivity and glucose tolerance can be improved in non-diabetic and diabetic subjects by weight loss and exercise\textsuperscript{121}. 
Different prospective studies demonstrated that a treatment regimen using diet and exercise reduces IGT progression to T2DM\textsuperscript{122, 123}. Even though, weight loss decreases the diabetes risk by 50-60%, about 40-50% of IGT subjects still progress to T2DM. This indicates that lifestyle intervention alone is not sufficient to prevent diabetes in a large percentage of individuals. In addition to behavioural intervention, pharmacological therapy (thiazolidinediones, metformin, GLP-1 receptor agonists etc) at the stage of IGT uniformly has been shown to effectively prevent IGT conversion to T2DM\textsuperscript{124}.

1. 10. 2 Insulin and oral therapies

Current therapies for diabetes have mainly based on elevating plasma insulin levels via direct insulin administration or oral agents that promote insulin secretion which improve insulin sensitivity of tissues and eventually reducing the rate of carbohydrate absorption from the gastrointestinal tract\textsuperscript{125}. The currently used drugs are sulfonylureas, glinides, GLP-1 receptor agonists, metformin, thiazolidinediones, and alpha-glucosidase inhibitors that target insulin resistance or beta-cell dysfunction by increasing insulin secretion or tissue insulin sensitivity\textsuperscript{125}. The history of diabetes medication is illustrated in Figure 1. 9.

1. 10. 2. 1 Insulin therapy

There were no effective pharmacological agents for the management of diabetes, until Banting et al. discovered the insulin therapy in 1921. The first commercial insulin was ‘Iletin’ developed by Eli Lilly in 1922. The next major advancement was crystallization of insulin in 1926 which improved purity of soluble insulin and also led to the insulin formulation modifications with different time-action profiles\textsuperscript{126}. In 1936, the first extended-action insulin, Protamine Zinc Insulin (PZI), composed of an amorphous combination of protamine, zinc, and insulin was released. In 1946, release of the second extended-action insulin, Neutral Protamine Hagedorn (NPH) by the Nordisk Insulin Laboratory which contained about 10% of the protamine found in PZI along with zinc insulin crystals\textsuperscript{126}. This insulin was shorter acting than PZI and could be combined with regular insulin. In 1956, the lente series of insulin was introduced: ultralente, lente, and semilente. These formulations were synthesized by altering the content of the excess zinc. All insulin preparations available before 1983 were derived from animal sources. By the
development of recombinant DNA technology, the first recombinant human insulin, got approval in 1983\textsuperscript{127}. The search for insulin that acts more closely with physiological insulin secretory patterns accelerated after the release of human insulin.

![Figure 1.9 The history of diabetes medications (reproduced from the reference 20 with permission).](image)

In the last years, increased advancement was noticed in the insulin preparations, i.e., the development of insulin analogs, which were designed to overcome the disadvantages of traditional human insulins in the treatment of both T1DM and T2DM. Insulin analogs more closely mimic the physiological insulin profile and are therefore associated with an improved balance between glycemic control and tolerability\textsuperscript{128}. Individuals with T1DM lack endogenous insulin and therefore rely entirely on injected insulin. The types of insulin available for current clinical use are rapid acting (bolus), long acting (basal) and intermediate types\textsuperscript{129}. Rapid-acting analogs, such as insulin aspart, insulin lispro, and insulin glulisine, are used as mealtime insulin replacement because they mimic the physiological insulin response to food intake\textsuperscript{130, 131}. It reduces the risk of hypoglycemia in the intervals between meals that is often seen with human insulin\textsuperscript{129, 130}. Long acting insulin analogues are insulin glargine and insulin detemir, which more closely
approximate the natural, constant physiological release of insulin\textsuperscript{130, 131}. Insulin glargine is injected as a solution, but precipitates upon injection into subcutaneous tissue, delaying absorption\textsuperscript{132, 133}. But, insulin detemir, forms hexamers and reversibly binds to albumin in the circulation, prolonging its absorption and bioavailability. The simultaneous supplementation of both prandial and basal insulin with a limited number of injections has been achieved the development of premixed insulin analogs\textsuperscript{130, 131} which are a mixed suspension of a rapid-acting analog along with its protamine-crystallized form. Several premixed insulin analogs are currently available, including biphasic insulin aspart (BIAsp 70/30; 30% rapid-acting insulin aspart and 70% protaminated insulin aspart) and biphasic insulin lispro (lispro mix 75/25; 25% rapid-acting lispro and 75% protaminated insulin lispro). The development of insulin pens and insulin pumps greatly improved the flexibility and convenience of insulin administration\textsuperscript{127, 134}. Results from large scale prospective studies such as the Diabetes Control and Complications Trial show that an intensive treatment regimen consisting of a rapid acting plus a long-acting insulin can help patients with T1DM to achieve better glycemic control, with a lower risk of complications, than less intensive therapy\textsuperscript{135}.

During the onset of type 2 diabetes, blood glucose levels can often be controlled with changes in diet and physical activity along with insulin secretagogues. But, the beta-cell dysfunction that leads to impaired insulin secretion is progressive, and eventually patients will require treatment strategies that include insulin, either alone or with oral agents\textsuperscript{136}. T2DM patients also develop both bolus (meal time) and basal (between meal) defects in insulin function, thus bolus and basal glucose levels are increased, resulting in hyperglycemia\textsuperscript{137}. So insulin therapy is recommended to these individuals by ADA. Intensive insulin therapy study in patients with T2DM showed that the insulin therapy partially reversed the post binding defect in peripheral insulin action, produced near-normal basal hepatic glucose output, and enhanced insulin secretion, thereby maintaining lower glucose values. In addition, the mean daily insulin requirement was reduced by 23% after about 2 weeks of therapy\textsuperscript{138}.

\textbf{1. 10. 2. 2 Metformin}

Metformin was developed from the antidiabetic folk remedy used in Southern and Eastern Europe called French lilac or goat’s rue, \textit{Galega officinalis}\textsuperscript{139}. The antihyperglycemic
moiety in this plant, ‘guanidine’ was isolated, in the early 20th century and later Frank et al. synthesized a guanidine compound called synthalin in Germany and used it to treat diabetes during the 1920s. Many synthetic analogues such as synthalin, phenformin and buformin were analysed by clinical trials, but due to hepatotoxicity and lactic acidosis led to their withdrawal from the market.

Metformin (1, 1-dimethyl biguanide), a biguanide derivative was introduced as an antihyperglycemic agent in 1959 but was approved in the United States only in 1990s. Nowadays, metformin is the most widely used antihyperglycemic agent in the world and is the only clinically significant biguanide. Metformin is recommended as the first-line oral therapy in the recent guidelines of the ADA (American Diabetes Association) and EASD (European Association of the Study of Diabetes). The main effect of this drug is to decrease hepatic glucose production through a mild inhibition of the mitochondrial respiratory chain complex I. This results in transient decrease in cellular energy status promotes the activation of AMPK, a well-known cellular energetic sensor. Thus, metformin-induced AMPK activation is believed to promote the transcriptional inhibition of the hepatic gluconeogenic programme. It also reduces plasma glucose via an increase in insulin-dependent glucose uptake. This medication has got a good tolerance in both humans and animal models and is typically associated with a significant reduction in % HbA1c levels, about 1.5%.

1. 10. 2. 3 Sulfonylureas

Sulfonylurea has been used in the treatment for type 2 diabetes for >50 years. Sulfonylureas increase the insulin secretion from pancreatic beta-cells by binding to receptors that block the K+-ATP-dependent channels, leading to cell depolarization and subsequently insulin exocytosis. Chlorpropamide, acetohexamide, and tolazamide were the first-generation sulfonylureas. In 1984, glyburide and glipizide, which are more potent second-generation sulfonylureas, became available in the market. Glimepiride, a third-generation sulfonylurea, was introduced in 1995. Sulfonylureas are cheap, and predictable, but the incidence of hypoglycemia, a major side effect, limits their use. The % HbA1C is decreased by 1-2%, by the administration of sulfonylureas.
1. 10. 2. 4 Thiazolidinediones

Thiazolidinediones (TZDs), or ‘glitazones’ were introduced to the U.S. market in 1996. TZD are peroxisome proliferator activated receptor-gamma (PPAR gamma) activators whose mechanisms of action are enhancement of skeletal muscle insulin sensitivity and reduction in hepatic glucose production. TZDs do not increase the risk of hypoglycemia and have a more durable effect than metformin or SUs and associated with an A1C decrease of 0.5-1% in most patients. Troglitazone was the first FDA approved TZD. But, the use of troglitazone was associated with hepatic failure, so FDA removed the drug from the market in 2000. The TZD which is currently available in the market is pioglitazone. Pioglitazone has been beneficial for cardiovascular disease moderately but it increases the incidence of bladder cancer. Even though, the TZD drug rosiglitazone acted as an effective insulin sensitizer, due to the increased risk of myocardial infarction (MI) and fluid retention there is a restriction for its use by FDA. The RECORD (Rosiglitazone Evaluated for Cardiovascular Outcomes and Regulation of Glycemia in Diabetes) study findings unveiled that people treated with rosiglitazone did not have an elevated risk of MI compared to patients taking other antihyperglycemic medications.

1. 10. 2. 5 Alpha glucosidase inhibitors

Alpha glucosidase inhibitors (AGIs) bring about a site specific effect in the small intestine, inhibiting alpha glucosidase enzymes resides in the brush border cells. Alpha-glucosidase is a collective term referring to membrane-bound enzymes of the small intestinal villi involved in the breakdown of alpha-linkages of oligosaccharides and disaccharides into glucose. The enzymes included are maltase, isomaltase, glucoamylase, and sucrase. AGIs reduce the rate of absorption of carbohydrates by prolonging the absorption time but do not alter the absolute absorption. Thus AGIs slow down the elevation of postprandial glucose levels. The reduction of HbA1c observed with AGIs is typically 0.5-1.0%. AGI drugs available in market are acarbose, miglitol and voglibose. Besides the slowing down of carbohydrate absorption, AGIs also augment incretin hormone secretion and enhances beta-cell function; by altering gut microbiota flora. AGIs also cause inhibition of platelet aggregation, attachment of macrophage to
vascular endothelium, ameliorate the development of atherosclerosis and help to maintain elasticity of blood vessels\textsuperscript{153}.

1. 10. 2. 6 GLP-1 mimetics or analogues and DPP-IV inhibitors

GLP-1, the hormone secreted by the L cells of the small intestines within minutes of carbohydrate or lipid rich meal stimulates insulin synthesis and glucose dependent insulin secretion\textsuperscript{154, 155}. GLP-1 also suppresses glucagon release and delays gastric emptying\textsuperscript{154, 155}. It has a short half-life of 1-2 min because of rapid degradation by the enzyme dipeptidyl peptidase-IV (DPP-IV)\textsuperscript{155}. Due to this physiological importance, the GLP-1 receptor agonists and DPP-IV inhibitors have been emerged as the treatment options for T2DM\textsuperscript{156}.

The first GLP-1 agonist introduced in the market is exenatide. It is a mimetic of exendin-4, a peptide isolated from the saliva of the \textit{Heloderma suspectum} (Gila monster), and has a 53\% similarity to the human GLP-1\textsuperscript{157, 158}. It is more resistant to DPP-IV degradation, thus has a longer half-life\textsuperscript{158}. Exenatide injection, twice daily, given 40-60 min before breakfast and dinner was approved in 2005\textsuperscript{159}. In 2012, a once-weekly formulation of exenatide (long acting release) was approved\textsuperscript{160}. A modified form of human GLP-1, with 97\% homology named liraglutide was approved in 2010 as daily injection\textsuperscript{161}. About 1\% decrease of HbA1c level was attained by GLP-1 receptor agonists. The gastrointestinal disorders and pancreatitis are reported in some patients\textsuperscript{162, 163}. There are three approved DPP-IV inhibitors: sitagliptin, saxagliptin, and linagliptin. The HbA1C reduction exhibited by DPP-IV inhibitors is up to 0.8\%. These are administered alone or in combination with metformin. The most attractive feature of GLP-1 agonist or DPP-IV is the absence of hypoglycaemia\textsuperscript{164-166}.

1. 10. 2. 7 Aldose reductase inhibitor

Aldose reductase inhibitors (ARIs) can block the metabolism of the polyol pathway and significantly reduces intracellular sorbitol accumulation in tissues where glucose uptake is independent of insulin\textsuperscript{167}. Hence it is used to slow or reverse the progression of diabetic secondary complications like retinopathy, neuropathy or nephropathy\textsuperscript{168}. Epalrestat, ranirestat, fidarestat are the currently available ARIs for the clinical use.
1. 10. 2. 8 SGLT-2 inhibitors

The sodium / glucose co-transporter 2 (SGLT-2) are high-capacity, low-affinity glucose transporter found primarily in the kidney\textsuperscript{169}. This transporter is responsible for about 90% of glucose reabsorption in the kidney\textsuperscript{169}. If SGLT-2 inhibitors are used, excess glucose in the renal tubules is not reabsorbed, and glucose is excreted in the urine. The result is the net loss of glucose and a reduction in hyperglycemia. Canagliflozin and Dapagliflozin A are the FDA approved SGLT-2 inhibitors\textsuperscript{170}. Recent meta-analysis studies evaluating SGLT-2 inhibitors reported HbA1C reductions of 0.5-0.6\% in patients treated with these agents\textsuperscript{171}.

1. 10. 2. 9 Emerging targets - AGE inhibitors, SIRT-1 activators, PTP-1B inhibitors

AGEs and AGE precursors damages the cells in several ways and cause secondary complication; by modifying the structure and function of transcription factors, components of extracellular matrix, circulating proteins. The circulating proteins with modified structure can then bind to AGE receptors (RAGEs) and activate them, thereby causing the production of inflammatory cytokines and growth factors, which in turn cause vascular pathology\textsuperscript{172, 173}. \textit{In vivo} studies have been shown that, pharmacologic inhibition of AGEs prevents late structural changes of experimental diabetic secondary complication such as retinopathy\textsuperscript{174}. The example for the inhibitors of renal AGE accumulation under clinical trial is alagebrium which is a specific AGE cross-link breaker\textsuperscript{175}.

SIRT-1 is a conserved NAD-dependent protein deacetylase. SIRT-1 influences several metabolic processes such as lipid and glucose metabolism, insulin signalling, suppression of the PTP-1B gene and others involved in energy maintenance and also regulate transcription coactivator PPAR-gamma coactivator-1 alpha or PGC-1alpha\textsuperscript{176}.

Protein tyrosine phosphorylation is a fundamental mechanism for the intracellular control of cell growth and differentiation. It is governed by the opposing activities of protein tyrosine kinases, which catalyze phosphorylation, and protein tyrosine phosphatases (PTPs), which are responsible for dephosphorylation\textsuperscript{125}. PTP-1B also dephosphorylates thereby inactivates (down-regulation) insulin receptors and insulin receptor substrates\textsuperscript{177, 178}. PTP-1B inhibition represents a therapeutic approach for insulin resistance and obesity.
Introduction

in T2DM\textsuperscript{179, 180}.

1. 10. 3 Traditional remedies for diabetes – role of Ayurveda

The human use of plants as medicines started in ancient times at least to the Middle Paleolithic age some 60,000 years ago\textsuperscript{181}. Traditional medicine has been defined by WHO as “diverse health practices, approaches, knowledge and beliefs incorporating plant animal or mineral based medicines, spiritual therapies, manual techniques or exercise applied singularly or in combination to maintain well being as well as to treat, diagnose or prevent illness”\textsuperscript{182}. According to WHO, almost 65% of the world’s population depends on complementary or alternative system for primary health care\textsuperscript{183}. Ayurveda is one of the oldest, health traditions in the world, originated in India. It is based on Sankhya philosophy, which means ‘rational enquiry into the nature of the truth’. Sanskrit meaning of Ayu is life and Veda is knowledge or science\textsuperscript{184}. Charak Samhita (1000 BC) and Sushrut Samhita (100 AD) are the main classics. Ayurveda materia medica gives detailed descriptions of over 1500 herbs and 10,000 formulations\textsuperscript{184}. Diabetes was known to ancient Ayurvedic physicians as Madumeha (madhu means ‘honey’ and meha means ‘urine’) and has given an elaborate description about this disease\textsuperscript{19}. Many herbs and herbal preparations in the form of kashayam or kwadha (decoction), rasayanam, bhasmam etc have been used to treat diabetes. WHO expert committee on diabetes has been recommended for the promotion of traditional methods for the treatment of diabetes\textsuperscript{185, 186}. A reverse pharmacology approach, inspired by traditional medicine and Ayurveda, can offer a smart strategy for new drug candidates to facilitate discovery process and also for the development of rational synergistic botanical formulations\textsuperscript{187}.

1. 11 Diabetes research tools for traditional plant based medications

The test material can be analysed for its antidiabetic effects using \textit{in vivo} and \textit{in vitro} test systems. Preliminary testing of the study material under \textit{in vivo} conditions can give valuable information on the type of extract to be made, a suitable dose, toxic effects, as well as it also helps to confirm efficacy. \textit{In vitro} tests can play an important role in the elucidating the molecular mechanism of the test material\textsuperscript{28}.
1.11.1 In vitro models for diabetes

In vitro assays rely on a specific biological process relevant to the disease and its treatment. Perfused whole organs, isolated tissues, cells in primary or immortal culture, subcellular membranes or purified receptors, and enzymes are the in vitro systems generally used. The advantages of in vitro assays in ethnobotanical research are reduced variability, low cost, minimal use of animals, it needed only less amount of material and above all of these, the mechanism of action of the test material can be elucidated as it uses a simple system compared to whole organism\textsuperscript{28}. The major in vitro test methods to evaluate antidiabetic potential are the following:

**Antioxidant activity:** Antioxidant activities of test material with respect to various reactive radicals (ABTS, DPPH, hydroxyl etc.) can be screened in cell free systems to see the possible role of these materials to minimise reactive oxygen species induced complications in diabetes.

**Inhibition of carbohydrate-digesting enzymes:** Inhibition of carbohydrate digestive enzymes (alpha glucosidase) can be measured to check the potential of the test material to control postprandial hyperglycemia.

**Antiglycation assay:** In vitro antiglycation screening is recommended in recent years to have active antiglycation agents against protein glycation during hyperglycemia.

**Inhibition of aldose reductase:** Aldose reductase inhibitors are potential candidates in diabetic therapy due to serious issue of retinopathy in diabetes. So there is high demand for development of aldose reductase inhibitors and in vitro system can be utilised for the same.

**Models based on the liver as an insulin target tissue:** Hyperglycemia induced oxidative stress can be developed in in vitro system utilizing HepG 2 cell lines. This system could be used to evaluate potential of test material in reducing hyperglycemia induced oxidative stress which is one of the main causes for diabetes secondary complications.

**Models based on adipocytes as an insulin target tissue:** Adipogenesis can be evaluated to test the TZD like activity of test material. Various factors associated with adipogenesis
like TG content, activity of GPDH and DGAT1 can also be utilised for the evaluation of adipogenic potential. The mouse-derived 3T3-L1 fibroblast cell line provides an alternative to the use of freshly isolated primary adipocytes. The cells are commercially available in preadipocyte form and can be induced to differentiate into adipocytes by the inclusion of a glucocorticoid (e.g., dexamethasone), insulin and an agent that elevates intracellular cAMP (e.g., isobutyl methyl xanthine, IBMX) in the culture medium\textsuperscript{188}. The differentiation process, as well as glucose uptake, lipogenesis, and inhibition of lipolysis in differentiated state can be assessed in this cell line as these are mediated by insulin.

Models based on muscle as an insulin target tissue: The uptake and utilization of glucose in the muscle is under control of insulin. A rat skeletal muscle cell line, L6 may be used for the study of antidiabetic agents. Cells are obtained as myoblasts that are induced, by adjusting the medium, to differentiate into an alignment stage and then into fused myotubes. Glucose uptake assay using \textsuperscript{3}H-2-deoxyglucose is most sensitive to insulin in the myotubes, corresponding to an increase in muscle specific GLUT-4 transporters\textsuperscript{189}.

1. 11. 2 In vivo models for diabetes

The classical animal model employed by Banting and Best was pancreatectomy in dogs\textsuperscript{24}. Today, most of the experiments in diabetes are carried out in rodents, although some studies are still performed in larger animals. Due to the heterogeneity of diabetic conditions, single animal model that exhibit all the features of diabetes is not available. Hence, different in vivo models, each displaying a different characteristic of diabetic states can be selected for the study\textsuperscript{190}. Normal nondiabetic animals and animals with impaired glucose tolerance and insulin resistance (but not overt diabetes) have also been used to demonstrate antihyperglycemic and insulin sensitizing activities and to investigate the mode of action of antidiabetic test materials\textsuperscript{191}. There is a marked overlap between type 1 and 2 diabetes mellitus either in human beings or in animal models, so the in vivo models for both the types of this disease are described here\textsuperscript{192, 193}.

Pharmacological models: Streptozotocin (STZ) and alloxan are the most frequently used drugs for the generation of drug induced model and is useful for the study of multiple aspects of the disease. The cytotoxic action of these diabetogenic agents is mediated by reactive oxygen species, but the mechanism of action of both drugs are different\textsuperscript{194, 195}.
Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. The superoxide radicals undergo dismutation to hydrogen peroxide with a simultaneous elevation in cytosolic calcium concentration, which causes rapid destruction of pancreatic beta-cells\textsuperscript{196}. The most frequently used intravenous dose of alloxan in rats is 65 mg/kg\textsuperscript{194}. Streptozotocin enters the pancreatic beta-cell via a glucose transporter, GLUT-2 and causes alkylation of deoxyribonucleic acid (DNA). Then, STZ induces activation of poly adenosine diphosphate ribosylation and nitric oxide release. As a result of STZ action, pancreatic beta-cells are destroyed by necrosis\textsuperscript{197}. In adult rats, 60 mg/kg is the most common dose of STZ to induce insulin dependent diabetes\textsuperscript{198}.

\textit{Diet induced models:} The basic diet-induced models are the following: high-fat diet, high-carbohydrate diet, combined high-fat and high-carbohydrate diet, diet with a high content of NaCl and fructose etc. High fat intake of saturated fatty acids and cholesterol causes obesity, insulin resistance, hepatic steatosis and increased content of triglycerides in muscles in rats\textsuperscript{199, 200}. Administration of high-fructose and high-sucrose diets allows the studies on muscle and liver abnormalities in a state of insulin resistance\textsuperscript{201, 202}. In order to stimulate hypertension and metabolic syndrome in rats, diet manipulations with high concentration of NaCl and fructose are used\textsuperscript{203}.

\textit{Surgical models:} The induction of diabetes can also be done with the complete surgical removal of the pancreas. Some reports are available with surgical model to explore effects of natural products with animal species such as rats, pigs, dogs and primates\textsuperscript{178, 204}.

\textit{Genetic models:} Animal strains that spontaneously develop diabetes and displaying complex and heterogeneous characteristics of diabetes such as insulin resistance, obesity, dyslipidemia and hypertension are considered in this category. This provides valuable insights to study some events that are observed in human T2DM. Some strains like ob/ob mouse may maintain euglycemia due to a robust and persistent compensatory pancreatic beta-cell response, matching the insulin resistance with hyperinsulinemia. On the other hand, the diabetic C57BL/KsJ db/db mouse rapidly develops hyperglycemia since their pancreatic beta-cells are unable to maintain the high levels of insulin secretion required throughout life\textsuperscript{205}. Thus, food intake is important in determining the severity of the
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diabetic phenotype and restriction of energy intake reduces both the obesity and hyperglycemia seen in this strain of mice. Another example is the spontaneously diabetic Goto-Kakizaki rat which is a genetic lean model of T2DM originating from selective breeding over many generations of glucose-intolerant nondiabetic Wistar rats\textsuperscript{206}. The NOD mouse shows resemblance to type 1 hyperglycemia between 12 and 30 weeks of age, whereas in BB rats it occurs around 12 weeks of age\textsuperscript{205}.

\textit{Transgenic models}: The transgenic or knockout rodent models targeting proteins thought to play a key role in glucose metabolism are helpful in giving insights into gene regulation and development, pathogenesis and finding new targets and the treatment of disease\textsuperscript{207}. The tissue specific knockout mouse models allows further insight into the insulin action with respect to particular target tissues like muscle, adipose or liver\textsuperscript{208, 209}.

1. 12 Aims and Objectives of the study

Most of current therapies for diabetes and its complications were developed by serendipity. Emerging knowledge of key pathogenic mechanisms, such as the impairment of glucose-stimulated insulin secretion and the role of ‘lipotoxicity’ as a probable cause of hepatic and muscle resistance to insulin’s effects on glucose metabolism, has led to a host of new molecular drug targets\textsuperscript{74}. Moreover, in spite of the introduction of various antidiabetic agents, diabetes and its secondary complications continue to be a global epidemic and there is high demand for novel treatment options without much side effects. Today, drug discovery strategies based on natural products and traditional medicines are re-emerging as attractive options\textsuperscript{210}. Rationally designed, carefully standardized, synergistic traditional herbal formulations and botanical drug products with robust scientific evidence can also be alternatives\textsuperscript{184}. Traditional knowledge is excellent source of novel drugs because of the increased tolerance, low cost, minimum side effects, local availability as well as synergistic effects. Ayurveda is the ‘great Indian tradition’ of ancient therapeutic approach with sound philosophical and experimental basis\textsuperscript{187}. ‘Nishakathakadi Kashayam’ is an effective Ayurvedic preparation for diabetes as per Ayurvedic script ‘Sahasrayogam’\textsuperscript{211}. The ingredients of Nishakathakadi Kashayam are \textit{Curcuma longa, Strychnos potatorum, Ixora coccinia, Emblica officinalis, Symplocos cochinchinenisis, Aerva lanata, Salacia reticulate and Vetiveria zizanioid}. 

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Figure 1.10. The plant selected for the study - *Symplocos cochinchinensis* (Lour.) S. Moore.

In the present study, we selected *Symplocos cochinchinensis* (Lour.) S. Moore. from the family *Symplocaceae*, also known as *pachotti* in Malayalam or *lodhra* in Hindi, is widely distributed in tropical and subtropical areas in Asia, Oceania, and America (Figure 1. 10). The leaves and bark of *S. cochinchinensis* are reported to have antioxidant, antihyperglycemic, hypolipidemic and antidiabetic properties\(^{212-215}\). *S. cochinchinensis* is also used in *Ayurveda* for the treatment of various disorders, such as leprosy, tumors, diarrhea, dysentery, inflammation, and uterine disorders\(^{216}\). The leaves have been used as a vegetable salad along with cucumber juice for the treatment of diabetes\(^{217}\). Anti-HIV\(^{218}\), inhibitory activities against phosphodiesterase\(^{219}\), antimicrobial\(^{220}\), antiinflammatory\(^{221}\), and antitumor\(^{222}\) applications have also been reported. The phytochemical investigation of the genus *Symplocos* had resulted in the isolation of confusoside, trilobatin, betasitosterol, symplocoside, salineposide, benzoysalireposide, oleanolic acid, beta-amyrin, and stigmasterol\(^{223}\).
The increasing use of traditional therapies demands more scientifically sound evidence for the principles behind therapies and for effectiveness of medicines\textsuperscript{187}. Recent advancements in the analytical and biological sciences, along with innovations in genomics and proteomics can play an important role in validation of these therapies\textsuperscript{210,224}. Even though, \textit{S. cochinchinensis} is known traditionally for its antidiabetic property as a component of \textit{Nishakathakadi Kashayam}, the mechanism of action of its antidiabetic activity was not clear. So, we decided to carry out a thorough mechanistic analysis of its antidiabetic potential utilizing various \textit{in vitro} and \textit{in vivo} methods for the scientific validation of its traditional use.

The main aims and objectives of the present study are

1. To evaluate the effect of \textit{Symplocos cochinchinensis} on \textit{in vitro} druggable targets relevant to diabetes.
2. To study the action of \textit{Symplocos cochinchinensis} on hyperglycemia induced secondary complications in rat model of streptozotocin diabetes (type 1).
3. To analyze the possible molecular mechanism behind the protective property of \textit{Symplocos cochinchinensis} against peripheral tissue (liver, muscle and adipose) insulin resistance and dyslipidemia in diet induced \textit{in vivo rodent} model (type 2).

1.13 Societal impact of the study

Kerala, the land of rich biodiversity and traditional and indigenous knowledge is located in the windward side of Western Ghats. The region of Western Ghats situated in Kerala is the richest in all three levels of biodiversity such as ecosystem diversity, species diversity and genetic diversity\textsuperscript{225}. Thousands of medicinal plants with excellent therapeutic potential are available in this region. Nowadays, the possibilities of sustainable utilization of biodiversity and indigenous knowledge remain unexplored. Recently, National Rural Health Mission reported that the diabetes prevalence is about 27\% in Kerala\textsuperscript{35}, even though Kerala is state of high literacy and improved healthcare facilities. So, it is high time to develop low cost and effective medication as well as lifestyle intervention strategies to reduce the incidence and prevalence of this epidemic. The prevalence of
diabetes can be minimised to a certain extent by utilizing the biodiversity and indigenous knowledge. The plant selected for present study is widely available in Western Ghats and it is already reported to have antidiabetic properties. The study is expected to uncover more scientific information for its use in diabetes which may scientifically validate the traditional knowledge for universal acceptance and wider use. Altogether, the outcome of the present study can be incorporated for lead generation in future for control and management of diabetes.
References


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