2. Literature review

2.1. Diabetes Mellitus

Diabetes mellitus (DM) is basically a group of metabolic disorders that is characterized by an elevated level of blood glucose (hyperglycemia) and insufficiency in production or action of insulin produced by the pancreas [34]. Insulin is a protein (hormone) synthesized in beta cells of the pancreas in response to various stimuli such as glucose, sulphonylureas, and arginine however glucose is the major determinant [35]. Long term elevation in blood glucose levels is associated with macro- and micro-vascular complications leading to heart diseases, stroke, blindness and kidney diseases [36]. Sidewise to hyperglycemia, there are several other factors that play a greater role in the pathogenesis of diabetes such as hyperlipidemia and oxidative stress leading to the high risk of complications [37].

2.2. Types of DM

DM can be classified in different ways, but one form of classification is as follows [38]:

1. *Type I diabetes (T1D; Insulin dependent)* is due to immune-mediated beta-cells destruction, leading to insulin deficiency.
2. *Idiopathic diabetes* is the type-1 diabetes with no known etiologies and is strongly inherited.
3. *Type II diabetes (T2D; Non-Insulin dependent)* is due to insulin secretory defect and insulin resistance.
4. *Gestational diabetes mellitus* is any form of intolerance to glucose with onset or first recognition of pregnancy.

However, diabetes is mostly classified basically into two major types: T1D and T2D.

2.3. Pathophysiology of diabetes

Whenever we take the meal, there is a rise in blood glucose levels that stimulates insulin secretion resulting in an increase in glucose transportation, biotransformation and storage in muscles and fat tissues. In fasting conditions, the glucose in the blood is provided by the liver that is used by the brain, without any dependency on insulin. Besides the storage of glucose, insulin also inhibits the secretion of glucagon and lowers the concentration of serum fatty acids leading to a decline in liver glucose production [37]. Insufficiency in insulin production or resistance to insulin in the body results in reduced tissue uptake of glucose that results in intracellular hypoglycaemia and extracellular hyperglycemia. The intracellular hypoglycaemia causes glucogenesis and gluconeogenesis that leads to fats breakdown (causing diabetic ketoacidosis) and decreases protein synthesis and gamma globulins (causing cachexia, polyphagia, and impaired wound healing), while the extracellular hyperglycemia leads to hyperglycemic coma and osmotic diuresis [39] (**Figure 2.1**).

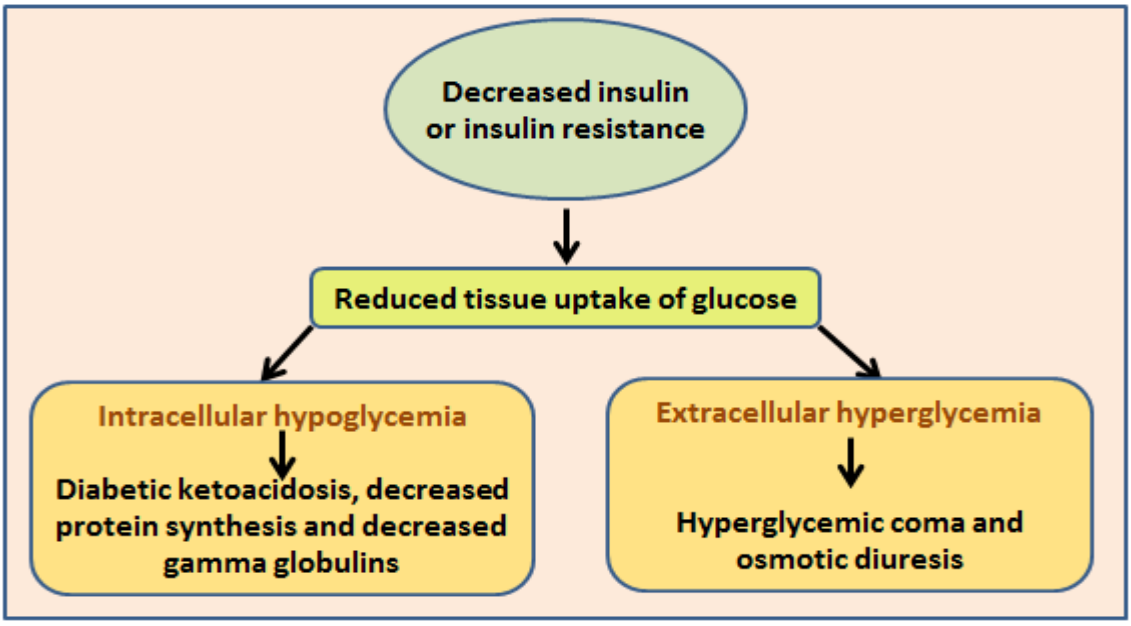


Figure 2.1: Pathophysiology of diabetes mellitus (modified from Ozougwu et al.) [39].

2.3.1. Pathogenesis of T1D

In T1D, there is a deficiency of insulin secretion due to the autoimmune destruction of pancreatic β cells that leads to metabolic disturbances associated with T1D [39]. The end-stage of β cell destruction represents the onset of clinical disease leading to T1D in which there are infiltrating monocytes, lymphocytes and a mixture of pseudoatrophic islets with some cells secreting somatostatin, glycogen and pancreatic polypeptide which then, consequently through the immunogenic process, induces the disease [40]. Autoimmunity, genetic makeup and environmental factors are responsible for islets cell destruction [41].

2.3.2. Pathogenesis of T2D

In T2D, there are certain mechanisms broken that keep regulation between tissue sensitivity to insulin which consequently leads to impaired insulin secretion by the pancreatic β cells and impaired insulin action through insulin resistance [42]. In this type of diabetes, multiple genetic defects, and certain environmental factors especially obesity are responsible for β cell defects and peripheral tissue insulin resistance respectively [41].

2.3.2.1. Aetiology

T2D is caused by a combination of genetic factors related to impaired insulin secretion and insulin resistance and environmental factors such as obesity, overeating, lack of exercise, and stress, as well as ageing. It is typically a multifactorial disease involving multiple genes and environmental factors to varying extents.

Genetic factors involved in the pathogenesis of diabetes

The development of T2D is clearly associated with a family history of diabetes. The significantly higher similarity rate between monozygotic twins than between dizygotic twins suggests the considerable involvement of genetic factors [43]. The pathogenesis has been assumed to involve a genetic abnormality in the molecules related to the regulatory system of glucose metabolism. The analysis of candidate genes targeted at glucose-stimulated insulin secretion of pancreatic β cells and the molecules comprising the molecular mechanism for insulin action have identified genetic abnormalities that can be independent causes of pathogenesis, including those in glucokinase genes, mitochondrial genes, and insulin receptor genes. Recently, a genome-wide association study (GWAS) has identified the mutation in the KCNQ1 gene related to insulin secretion abnormality as an important disease-susceptible gene associated with the pathogenesis of diabetes in Asian ethnic groups [44].

Roles of environmental factors

Ageing, obesity, alcohol drinking, smoking, etc. are independent risk factors of pathogenesis. Obesity (particularly visceral fat obesity) due to a lack of exercise is accompanied by a decrease in muscle mass, induces insulin resistance, and is closely associated with the rapid increase in the number of middle- and high-aged patients. The changes in dietary energy sources, particularly the increase in fat intake, the decrease in starch intake, the increase in the consumption of simple sugars, and the decrease in dietary fibre intake, contribute to obesity and cause deterioration of glucose tolerance. Even mild obesity (BMI<25) causes a 4- to 5-fold increase in the risk of developing diabetes if accompanied by the increase in visceral fat mass [44] (**Table 2.1**).

Table 2.1: Factors causing an increase in visceral fat [44]

S. no.	Factors
1	Stress-related factors: • Overeating, especially excessive intake of simple sugars • Smoking • Increase in alcohol intake • Disorders of nervous and endocrine systems: increase in cortisol, abnormality in sex hormone secretion
2	Lowered energy consumption due to a lack of exercise
3	Genetic factors
4	Ageing

2.3.2.2. Pathophysiology

Impaired insulin secretion and insulin resistance contribute more or less jointly to the development of pathophysiological conditions of T2D.

Impaired insulin secretion

Impaired insulin secretion is a decrease in glucose responsiveness, which is observed before the clinical onset of disease. More specifically, impaired glucose tolerance (IGT) is induced by a decrease in glucose-responsive early-phase insulin secretion, and a decrease in additional insulin secretion after meals causes postprandial hyperglycemia. An oral glucose tolerance test (OGTT) in IGT cases generally indicates an over-response in individuals, who have markedly high insulin resistance. Contrarily, the decrease in early-phase secretion is an essential part of this disease, and is extremely

important as a basic pathophysiological change during the onset of disease in all ethnic groups [45].

Impaired insulin secretion is generally progressive, and its progression involves glucose toxicity and lipo-toxicity. When untreated, these are known to cause a decrease in pancreatic β cell mass in experimental animals. The progression of the impairment of pancreatic β cell function greatly affects the long-term control of blood glucose. While patients in early stages after disease onset chiefly show an increase in postprandial blood glucose as a result of increased insulin resistance and decreased early-phase secretion, the progression of the deterioration of pancreatic β cell function subsequently causes permanent elevation of blood glucose [45].

Insulin resistance

Insulin resistance is a condition in which insulin in the body does not exert sufficient action proportional to its blood concentration. The impairment of insulin action in major target organs such as liver and muscles is a common pathophysiological feature of T2D. Insulin resistance develops and expands prior to disease onset. The investigation into the molecular mechanism for insulin action has clarified how insulin resistance is related to genetic factors and environmental factors (hyperglycemia, free fatty acids, inflammatory mechanism). Known genetic factors include not only insulin receptor and insulin receptor substrate (IRS)-1 gene polymorphisms that directly affect insulin signals but also polymorphisms of thrifty genes such as the β_3 adrenergic receptor gene and the uncoupling protein (UCP) gene, associated with visceral obesity and promote insulin resistance. Glucolipotoxicity and inflammatory mediators are also important as the mechanisms for impaired insulin secretion and insulin signalling impairment [46].

Recent attention has focused on the involvement of adipocyte-derived bioactive substances (adipokines) in insulin resistance. While TNF-α, leptin, resistin, and free fatty acids act to increase resistance, adiponectin improves resistance. Clinical tests to assess the extent of insulin resistance include homeostasis model assessment for insulin resistance (HOMA-IR), insulin sensitivity test (loading test), steady-state plasma glucose (SSPG), minimal model analysis, and insulin clamp technique. The Matsuda index is now gaining recognition as a relatively simple procedure that can simultaneously evaluate insulin resistance in the liver and muscles [46]. After performing OGTT, this index is calculated by the formula: Matsuda Index = 10,000/√(FPG×FPI) × (mean PG×mean PI), where FPG is fasting plasma glucose, and FPI is fasting plasma insulin. A more convenient way to estimate the degree of resistance is to check for the presence of high fasting blood insulin, visceral obesity, hypertriglyceridemia (**Figure 2.2**).

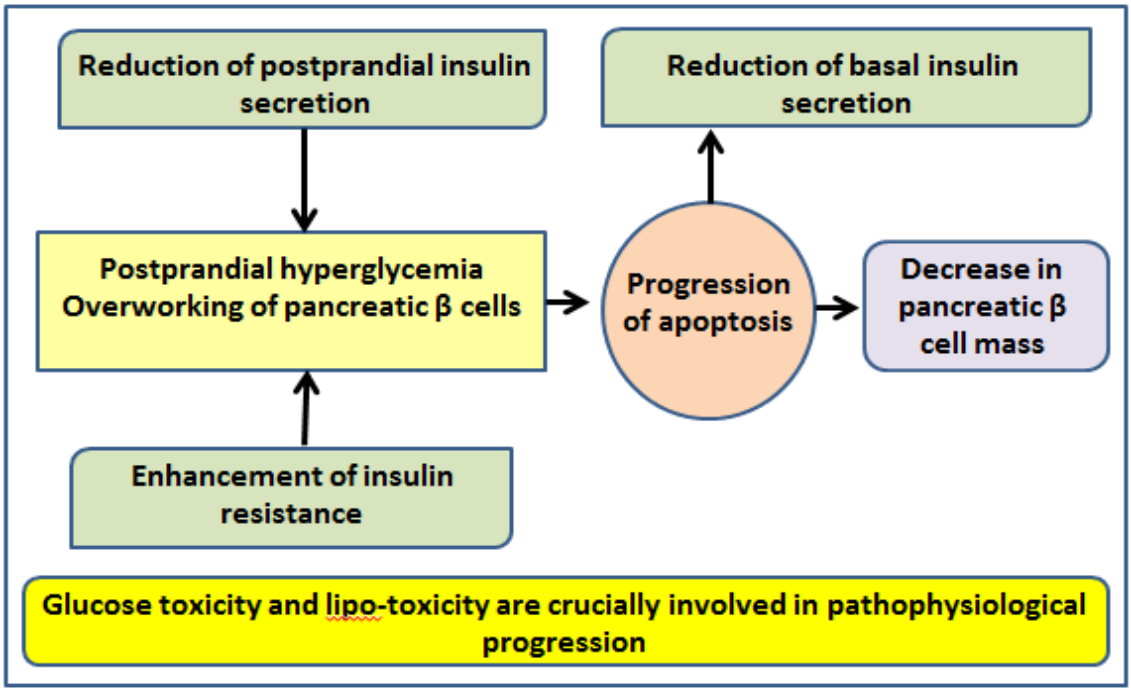


Figure 2.2: Pathophysiological progression of T2D (modified from Matsuda et al.) [46].

2.4. The management paradigm for T2D

Early initiation of intervention is also important for curbing the progression of pathophysiological conditions. Early efforts to remove the effect of glucose toxicity as much as possible and to preserve pancreatic β cell function are essential prerequisites for long-term management of diabetes. Microvascular disease is more closely associated with long-term blood glucose control. The treatment paradigm needs to be considered from the viewpoints of not only controlling vascular complications but also preventing the progression of pathophysiological conditions. In this sense, it is necessary to move up the treatment schedule (**Figure 2.3**). Ideally, the aim should be to prevent the onset of diabetes among individuals with IGT (primary prevention). In addition to proactive intervention for lifestyle improvement, we need to accelerate the debate about whether to use pharmacological intervention [47].

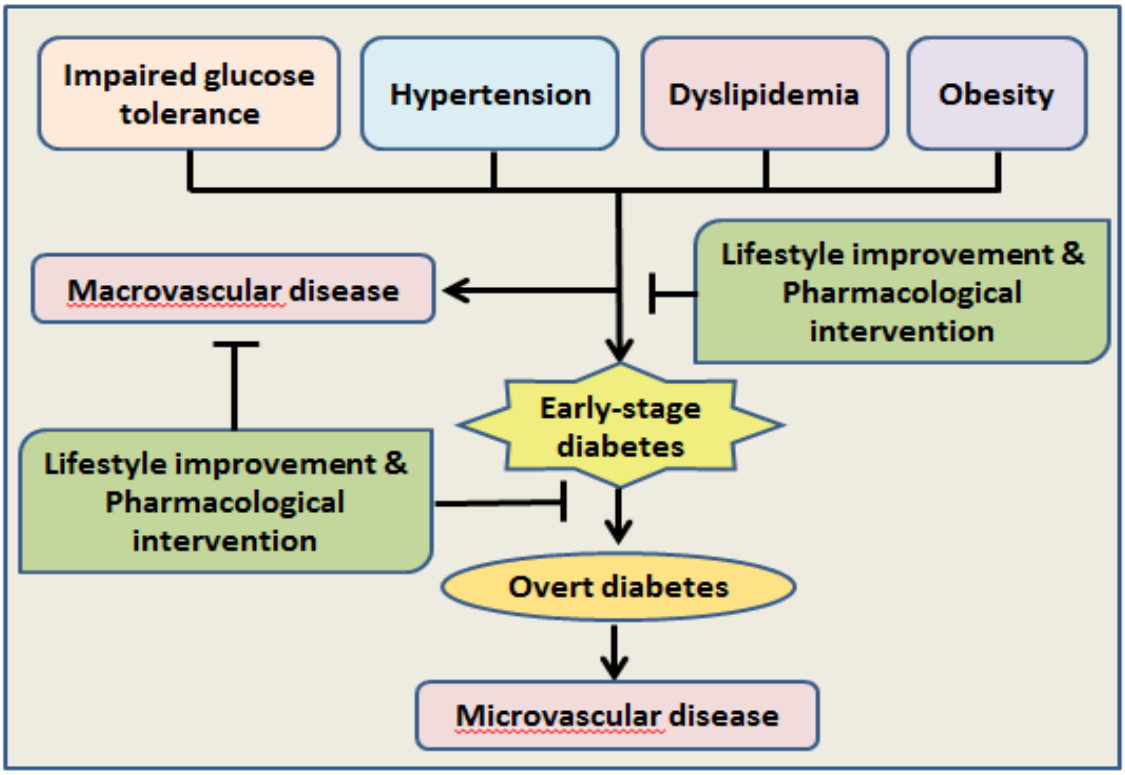


Figure 2.3: The management paradigm for T2D: prevention of onset and proactive management of early-stage diabetes (modified from Chiasson et al.) [47].

2.5. Epidemiology of diabetes

According to the International Diabetes Federation, there were an estimated 382 million people affected with diabetes globally in 2013, expected to reach 592 million by 2035 (**Table 2.2**). The burden of diabetes is rising rapidly in every country, fuelled by the worldwide increase in the incidence of obesity and unhealthy dietary habits.

Table 2.2: Top ten countries for number of adult population (aged 20–79 years) with diabetes in 2013 and 2035

S.No.	Year			
	2013		2035	
	Country	No. of adults with diabetes (millions)	Country	No. of adults with diabetes (millions)
1	China	98.4	China	142.7
2	India	65.1	India	109.0
3	USA	24.4	USA	29.7
4	Brazil	11.9	Brazil	19.2
5	Russian Federation	10.9	Mexico	15.7
6	Mexico	8.7	Indonesia	14.1
7	Indonesia	8.5	Egypt	13.1
8	Germany	7.6	Pakistan	12.8
9	Egypt	7.5	Turkey	11.8
10	Japan	7.2	Russian Federation	11.2

Adapted from International Diabetes Federation, Diabetes Atlas (Sixth Edition) (www.idf.org/diabetesatlas), 2013, Brussels.

T1D and T2D are widely accepted aetiological forms of diabetes; however, T2D is responsible for the majority (>85%) of the total prevalence of diabetes. The exact time of onset of T2D is hard to determine, and up to one-half of diabetic patients remain undiagnosed because of its slow onset nature and the absence of acute metabolic disturbance seen in T1D. Furthermore, different factors like genetic predisposition, ethnicity, lifestyle changes, and globalization contribute to the rapid prevalence of diabetes in India. Indian Council of Medical Research (ICMR) conducted an extensive community study and reported that a less population is affected in states of Northern India (Jharkhand 0.96 million, Chandigarh 0.12 million) as compared to Tamil Nadu (4.8 million) and Maharashtra (9.2 million) [48]. Thus, there is a noticeable geographical variation in the prevalence of diabetes, but with a different pattern.

Two population-based studies like CURES (Chennai Urban Rural Epidemiology Study) [49] and CUPS (Chennai Urban Population Study) [50] provide a clear cut idea about the prevalence of diabetic complications in India. In the CURES study, the overall prevalence of diabetic retinopathy, overt nephropathy, microalbuminuria, proteinuria and peripheral neuropathy were 17.6% [51], 2.2% [52], 26.9% [52], 19.7% [53] and 26.1% [54] respectively. Thus, the increased morbidity and mortality in diabetic patients could be attributed to an increased burden of diabetic macro- and microvascular complications. Therefore, the morbidity, mortality, increased financial burden, inadequate health care, and reduced life expectancy make diabetes a significant public health condition.

2.6. Complications of diabetes

Diabetes is such a sort of disorder in which the patients are at all the time on risk of complications. Complications may be macrovascular (coronary heart disease, peripheral

vascular disease and stroke), microvascular (neuropathy, retinopathy and nephropathy) and both micro- and macrovascular (diabetic foot). The mortality and morbidity of diabetes are associated more with macrovascular degeneration as compared to the risks of microvascular complications in older people [55]. In general, complications of DM can be categorized into two groups [55, 56]:

a. Metabolic acute complications: These are short term and include hypoglycemia, ketoacidosis and hyperosmolar non-ketonic coma.

b. Systemic late complications: These are long term chronic sort of complications that include diabetic nephropathy, microangiopathy, diabetic neuro- and retinopathy, atherosclerosis and infections.

2.7. Diabetic nephropathy

Diabetic nephropathy (DN) or diabetic kidney disease is a syndrome characterized by the presence of pathological quantities of urine albumin excretion, diabetic glomerular lesions, and loss of glomerular filtration rate (GFR) in diabetics. The estimated glomerular filtration rate (eGFR) may progressively fall from a normal of over 90 ml/min/1.73m^2 to less than 15, at this point the patient is said to have end-stage kidney disease (ESKD) and DN usually progresses slowly over the years [57].

2.8. Epidemiology of DN

The average incidence of diabetic nephropathy is high (3% per year) during the first 10 to 20 years after diabetes onset [58]. Typically, it takes 15 years for small blood vessels in organs like kidney, eyes and nerves to get affected. It is estimated that more than 20 and up to 40% of diabetic patients will develop chronic kidney disease (CKD) [59, 60], depending upon the population, with a significant number that develop ESKD requiring

renal replacement therapies such as kidney transplantation. Incidentally, diabetes with no clinical sign of kidney damage during the initial 20 to 25 years is significantly less likely (1% a year) to cause major renal complication later in life [58].

2.9. Pathological stages of DN

The different stages of DN are, as represented in **Table 2.3**.

Table 2.3: Staging of DN

Stages	DN staging Tervaert et al. [61]	DN staging Gheith et al. [62]
Stage 1	Glomerular basement membrane thickening	From onset to 5 years: Borderline GFR, no albuminuria, hypertension. But kidney size increased by 20% along with an increase in the renal plasma flow
Stage 2	Mild or severe mesangial expansion	From 2 years after onset with basement membrane thickening and mesangial proliferation, normal GFR and no clinical symptoms
Stage 3	Nodular sclerosis	5–10 years after onset with or without hypertension, with glomerular damage and microalbuminuria (30–300 mg/day)
Stage 4	Advanced diabetic glomerulo-sclerosis that includes tubulointerstitial lesions and vascular lesions	Irreversible proteinuria, sustained hypertension and GFR below 60 ml/min/1.73 m^2
Stage 5	-----	End-stage kidney disease with GFR < 15 ml/min/1.73 m^2

2.10. Pathophysiology of DN

DN is basically characterized by structural and functional changes in the kidney. In glomeruli, there is mesangial expansion, thickening of the basement membrane and, characteristically nodular glomerulosclerosis (Kimmelstiel–Wilson nodules). In early DN, tubular hypertrophy is present but eventually, interstitial fibrosis with tubular atrophy develops, along with arteriolar hyalinosis. In advanced cases, there is an infiltration of macrophages and T-lymphocytes. Ultrastructurally, there is podocyte loss and reduced endothelial cell fenestration [63]. Functionally, there is early glomerular hyperfiltration and increased albumin excretion; and with advancing nephropathy, increasing proteinuria and declining GFR [64]. Although it is conceptually easier to describe these pathways individually, these pathways overlap and interact with one another *in vivo*, and enhance one another's physiological effects (**Figure 2.4**).

2.10.1. Metabolic factors involved in DN pathogenesis

Oxidative stress and generation of reactive oxygen species (ROS) damage DNA and protein, or function as signalling amplifiers to activate cellular stress pathways such as PKC, MAPK and NF-κB [65]. Activation of the polyol pathway with aldose reductase converting excess glucose to sorbitol and subsequent conversion to fructose by sorbitol dehydrogenase contributes to oxidative stress by increasing the NADH/NAD+ ratio [66]. A recently described novel mechanism of injury also involves endogenous fructose production with activation of fructokinase in the proximal tubule. The formation of advanced glycation end-products (AGE) by nonenzymatic binding of glucose to proteins, lipids, and nucleic acids can lead to alteration of protein structure and function, oxidative stress, and expression of proinflammatory cytokines and growth factors [67].

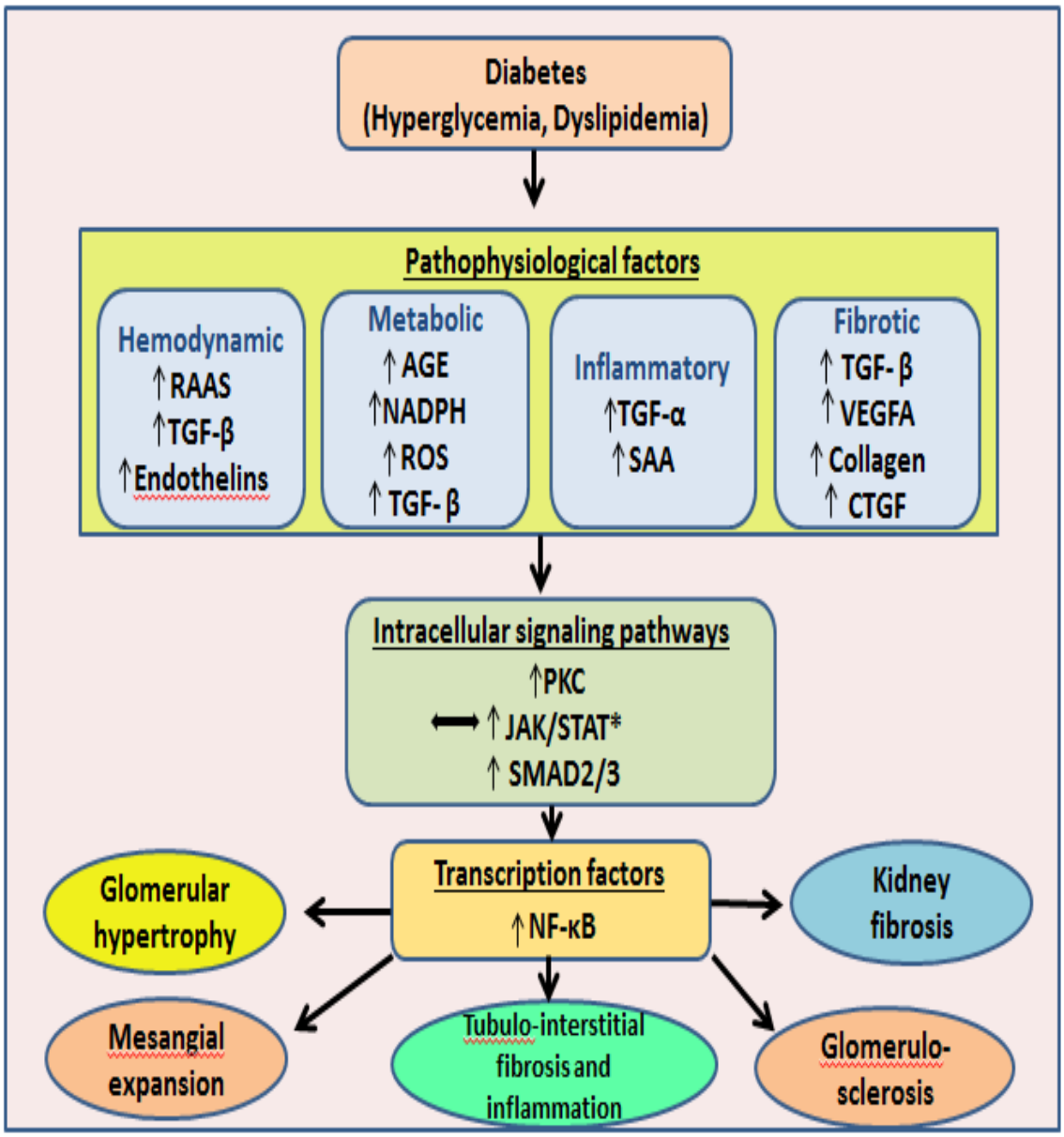


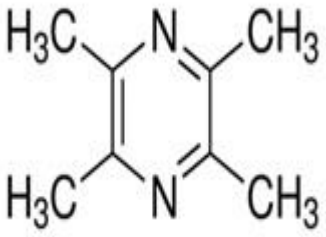
Figure 2.4: Different pathways and networks involved in the initiation and progression of DN. AGE, advanced glycation end product; CTGF, connective tissue growth factor; JAK/STAT, Janus kinase/signal transducer and activator of transcription; PKC, protein kinase C; RAAS, renin-angiotensin-aldosterone system; ROS, reactive oxygen species; SAA, serum amyloid A; VEGF-A, vascular endothelial growth factor A. *JAK/STAT signalling can be unchanged (↔) or upregulated (↑) in early and later stages of diabetes, respectively (modified from Toyoda et al.) [64].

2.11. Tetramethylpyrazine

2.11.1. Source and history

Tetramethylpyrazine (TMP), also known as *ligustrazine*, is a biologically active alkaloid (**Table 2.4**) isolated from the traditional medicinal plant *Ligusticum wallichii Franch*. It has long been used in clinical practice to improve circulation and prevent clot formation [68]. It is well known that TMP acts as a calcium channel antagonist [69] or as an antioxidant [70]. Recently, TMP has received attention for its distinctive roles in stimulating neurogenesis after focal ischemia in the rat brain [21], inducing neuronal differentiation of rat neural stem cells [71], displaying neuroprotective roles in traumatic spinal cord injury [72] and chronic hypoxia of the medulla oblongata in a rat model [73], in addition to improving scopolamine-induced memory impairment [74]. TMP has also been applied in the treatment of stroke and cardiovascular diseases for a long time in Oriental medicine. Some previous studies have shown that TMP has vasodilatory and antihypertensive effects [75]. It may also reduce oxidative stress and create endothelial protection [23]. In traditional clinical practice, TMP has been found to improve microcirculation, correct hypercoagulability, and promote vascular recanalization. Few earlier studies also reported that TMP has an ameliorating effect in diabetic nephropathy [33].

Table 2.4: TMP structure and formula

Structure	
Molecular weight	136.19 g/mol
Chemical formula	$C_8H_{12}N_2$
IUPAC name	2,3,5,6-Tetramethylpyrazine
Other names	Ligustrazine, Tetrapyrazine

2.11.2. Therapeutic activity of TMP in various diseases

2.11.2.1. TMP induces neuronal differentiation in neurodegenerative diseases

Recently, it was demonstrated that SH-SY5Y cells treated with TMP (80 μM) terminally differentiated into neurons, characterized by increased neuronal markers, tubulin βIII and microtubule associated protein2 (MAP2), and increased neurite outgrowth, with no negative effect on cell survival. TMP also increased the expression of TopoIIβ, which was accompanied by increased expression of specificity protein 1 (Sp1) in the differentiated neuron-like cells, whereas nuclear factor Y (NF-Y) protein levels remained unchanged following the differentiation progression. It was also found that the phosphorylation level of Akt, but not ERK1/2, was significantly increased as a result of TMP stimulation. Furthermore, as established by chromatin immunoprecipitation (ChIP) assay, activation of the PI3K/Akt pathway increased Sp1 binding to the promoter of the TopoIIβ gene. Blockage of PI3K/Akt was shown to lead to subsequent inhibition of TopoIIβ expression and neuronal differentiation. Collectively, the results indicated that the PI3K/Akt/Sp1/TopoIIβ signalling pathway is

necessary for TMP-induced neuronal differentiation. It suggests potential applications of TMP both in neuroscience research and clinical practice to treat relevant diseases of the nervous system [22].

2.11.2.2. TMP protects against vascular complications of diabetes

One of the earlier studies showed that TMP could protect endothelial cells from high glucose-induced damages, such as ROS production, down-regulation of Akt/eNOS phosphorylation and reduction of NO generation. The protective actions of TMP were partially attributed to uncoupling protein 2 (UCP2) mRNA/protein expression because the silence of a UCP2 gene by siRNAs (small interfering RNAs) abolished such effects. All of the data, taken together, points to the therapeutic potential of TMP for vascular complications of diabetes [23].

2.11.2.3. TMP ameliorates DN

In one of the earlier investigations, the protective effect of TMP on diabetic nephropathy in a rat model was studied and the possible mechanism involved in a protective function was also explored. Diabetes was induced in male Sprague-Dawley rats by a single intraperitoneal injection (i.p.) of 70 mg/kg of streptozotocin (STZ). One week later, 200 mg/kg/day of TMP was administered by intragastric gavage daily for 8 weeks. Renal functions and expression of vascular endothelial growth factor (VEGF) were examined at 4 and 8 weeks after TMP administration. In this study, STZ-induced diabetes resulted in significant renal damage with abnormal 24-h urinary protein and creatinine clearance rates. TMP treatment had a protective effect on the kidney in this diabetes model. Results of the study suggested that the expression of VEGF may be

involved in the protective mechanism of TMP against diabetic nephropathy and that TMP may be beneficial for the clinical treatment of diabetic nephropathy [33].

2.12. Role of PI3K/Akt/GLUT-4 signalling in insulin-mediated glucose uptake and amelioration of insulin resistance

2.12.1. Insulin signalling pathway

Insulin released from the β cells of pancreas participates in many metabolic actions, such as glycogen deposition in liver and skeletal muscles, stimulation of lipogenesis and inhibition of lipolysis, and repression of gluconeogenesis in the liver, but mainly in increasing glucose uptake through insulin receptor signalling pathway [76]. Signal transmission from the blood to the inside of the cell is a complicated and strongly integrated process. It begins with the binding of the hormone to the insulin receptor (IR), eliciting the large protein signal complex formation just below the surface of the cell membrane around IR's cytoplasmic domains (**Figure 2.5**) [77]. IRs are heterotetrameric glycoproteins containing two extracellular (α) and two intracellular (β) subunits. They occur mainly on the cell surface of metabolically active tissues like muscles, liver, and fat. The binding of insulin by extracellular subunits leads to IR dimerisation, which allows ATP binding to β-subunits. This causes the activation of the catalytic domains of tyrosine kinases in the cytoplasm. In the first stage, there is an autophosphorylation of the receptor, followed by phosphorylation of several substrate proteins, where IRS proteins seem to be the most significant ones. The phosphorylation occurs on tyrosine residues, and then, phosphorylated IRS proteins can trigger two major signalling pathways. The first pathway leads from Ras to mitogen-activated kinases (MAPK), being involved in the expression of genes playing a role in cell growth and differentiation. The second one, phosphatidylinositol 3 kinase (PI3K) pathway,

elicits AKT/PKB kinase phosphorylation, and it is responsible for the metabolic action of insulin [78].

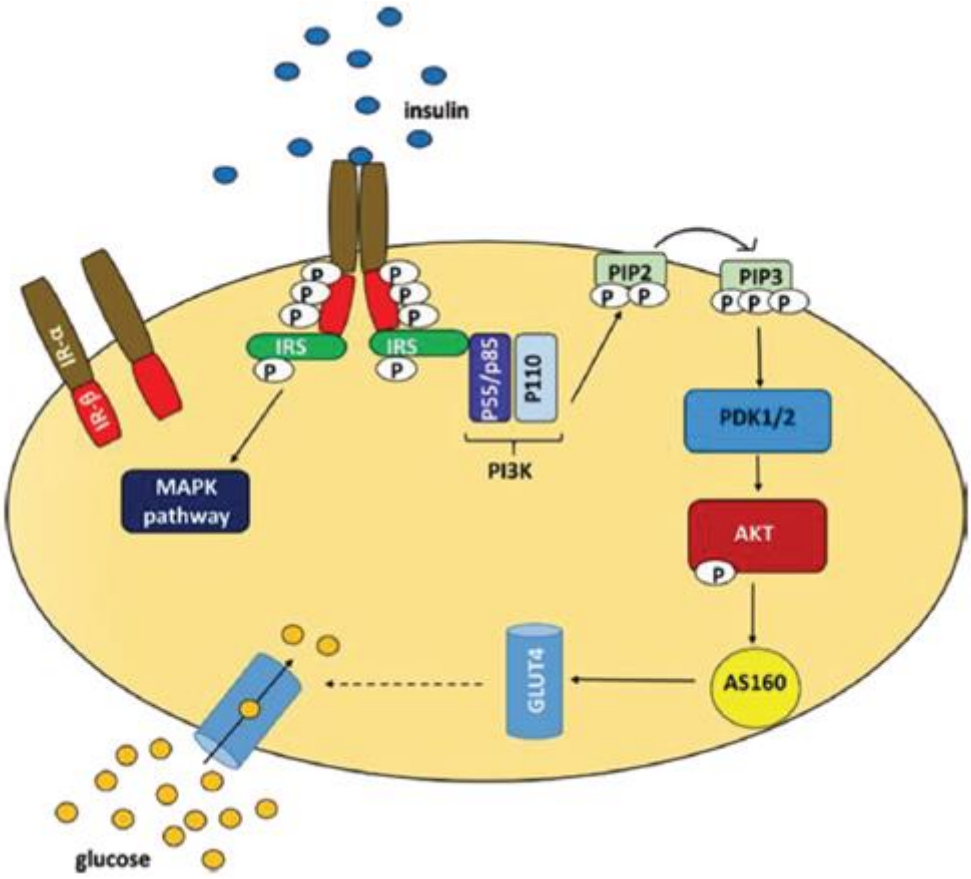


Figure 2.5: Insulin signalling pathway. Insulin attaches to insulin receptors triggering its dimerisation and intracellular autophosphorylation of their tyrosine residues, which constitute an attachment for IRS proteins. These molecules also undergo phosphorylation and form a complex with PI3K utilising SH2 domains. PI3K phosphorylates PIP2, which results in PIP3 formation and activation of PDK1/2. AKT gets phosphorylated and activated by PDK1/2, subsequently eliciting phosphorylation of AS160. The latter is responsible for GLUT-4 translocation to the cellular membrane and glucose inflow [77].

2.12.2. The PI3K/AKT pathway

As shown in **Figure 2.5**, activation of the PI3K/AKT pathway starts with the binding of IRS proteins via SH2 domains to PI3 kinase regulatory subunits. This results in the activation of PI3K that phosphorylates phosphatidylinositol 4,5-biphosphate (PIP2) to phosphatidylinositol (3,4,5)-triphosphate (PIP3). This, in turn, leads to the activation of

PIP3-dependent kinases: PDK-1 and PDK-2 and eventually to the activation of AKT/PKB kinase and atypical PKCs [79]. Subsequently, AKT catalyses the phosphorylation of AS160 substrate protein that stimulates the translocation of GLUT glucose transporters from the cytoplasmic vesicles onto the cell membrane surface and thereby increases the insulin-dependent transport of glucose into the cell. GLUT-4 occurs mainly in the interior of the nonstimulated cell, due to the proper proportion of two actions: slow exocytosis and rapid endocytosis. AS160 increases GLUT-4 exocytosis and inhibits its endocytosis via its downstream target, Rab10, in adipocytes. This results in GLUT-4 accumulation in the plasma membrane. Besides the activation of insulin-dependent glucose uptake via GLUT-4, AKT has many intracellular targets and mediates numerous metabolic effects. For instance, AKT triggers phosphorylation of glycogen synthase kinase 3 (GSK3), which leads to stimulation of glycogen synthesis in liver and skeletal muscle [80].

2.13. Role of Akt signalling in the amelioration of renal apoptosis in DN

Previous studies have confirmed that cell apoptosis plays a crucial role in renal structural and functional damage that follows the stimulus of high blood sugar, and the underlying mechanism of such injury and dysfunction are not well understood [81]. Nevertheless, Akt signalling has been reported as an important component involved in the pathogenesis of DN (**Figure 2.6**). The Akt phosphorylation can improve anti-apoptotic and antioxidant capacity in diabetic animals, but Akt phosphorylation can also be inhibited by oxidative stress [82]. When Akt phosphorylation is decreased, its downstream factors GSK-3β phosphorylation is activated, and other apoptosis-associated factors such as BAX and Cleaved Caspase-3 were increased [83].

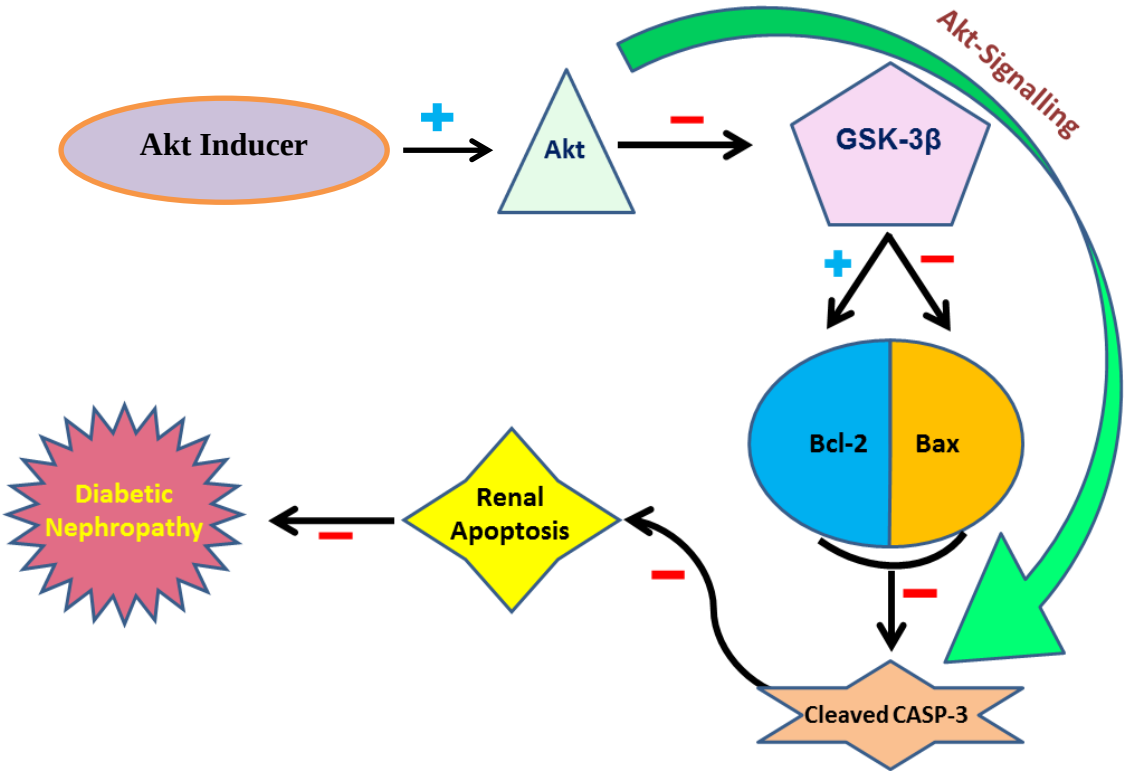


Figure 2.6: Role of Akt signalling pathway in the alleviation of renal apoptosis in DN.