1. INTRODUCTION

"Just when you think and feel you have got all the answers to all the questions in your life, God changes the question paper itself!"

- Sujit Lalwani, Founder & Chairman, Inspiration Unlimited

At the dawn of the third millennium, non-communicable diseases such as cardiovascular diseases, diabetes, cancer and mental disorders appeared to be sweeping the entire globe, with an increasing trend in developing countries. Globally, the proportion of the burden of diseases is shifting from communicable diseases to non-communicable diseases. If the present trend is maintained, it is estimated that, by 2020, non-communicable diseases will account for 80% of the global burden of diseases, causing seven out of every ten deaths in developing countries, compared with less than half today (WHO, 2002).

Most of the non-communicable diseases are preceded by unhealthy behaviors followed by the emergence of metabolic risk factors and onset of diseases. The major risk factors associated with non-communicable diseases include overweight, obesity, chronic hyperglycemia, hyperlipidemia which considered modifiable through changes in behaviors or medications. The key behaviors that would reduce the risk factors for non-communicable diseases are eating a healthy diet, participating in regular physical activity, not using tobacco and avoiding harmful use of alcohol. Among the non communicable diseases, diabetes mellitus is the most prevalent and pervasive metabolic
disorder next to cancer and its burden is exacerbated by micro and macro vascular complications leading to blindness, amputations, kidney failure and heart diseases.

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

Several pathogenic processes are implicated in the development of diabetes. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues (American Diabetes Association, 2012). Impaired insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia.

Symptoms of marked hyperglycemia include polyuria, polydipsia, unusual weight loss, polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic
hyperglycemia. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the non-ketotic hyperosmolar syndrome.

**PREVALENCE OF DIABETES MELLITUS**

The number of people with diabetes is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity. Quantifying the prevalence of diabetes and the number of people affected by diabetes, now and in the future is important to allow rational planning and allocation of resources. According to International Diabetes Federation (IDF) 382 million people worldwide, or 8.3% of adults, are estimated to have diabetes. About 80% of the diabetics are living in low- and middle-income countries. If this trend continues, by 2035, more than 592 million people, or one adult in 10, will have diabetes. This equates to approximately three new cases every 10 seconds or almost 10 million per year (IDF, 2013).

The largest increases will take place in the regions where developing economies are predominant. In high-income countries, most people with diabetes are aged over 50 years, whereas in middle-income countries, the highest prevalence is in younger people—the most productive age groups (Abegunde et al., 2007). As these people grew, and life expectancies increase, prevalence in older age groups will also rise further. This trend will pose a huge burden on healthcare systems.
Mortality is an important measure of population health and is often used to assign priorities in health care interventions. Estimating mortality due to diabetes has been challenging because more than one third of countries of the world have no reliable data available on mortality and also the existing routine health statistics have been shown to underestimate mortality from diabetes. The number of excess mortality due to diabetes was presented by IDF and it was predicted that almost 4 million deaths in 2010 could be attributed to diabetes, which is 6.8% of global (all ages) all-cause mortality.

In all regions, bar one, roughly 10% or more of deaths in the age group 20–79 were attributable to diabetes, with the highest proportion (15.7%) being in North America, reflecting both a high prevalence of diabetes and a relatively elderly population (IDF, 2007). Africa is the region with the lowest proportion of deaths attributable to diabetes in adults, wherein diabetes accounted for over 1 in 20 deaths.

Table 1: Top 10 countries with number of individuals with diabetes (20–79 years) in 2013 and 2035.

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SIGNS AND SYMPTOMS

The most common signs and symptoms of diabetes include polyuria (frequent urination), polydipsia (excessive thirst), polyphagia (excessive hunger), weight loss, and blurred vision (American Diabetes Association, 2012). The common symptoms include,

- Weight loss
- Blurry vision
- Irritability
- Nausea
- Frequent infections that are slow to heal
- Numbness and tingling in the feet
- Fatigue
- Skin diseases

Classification of Diabetes

According to American Diabetes Association, 2013, diabetes can be classified into four major clinical categories:

- **Type 1 diabetes** (due to β-cell destruction, usually leading to absolute insulin deficiency)
- **Type 2 diabetes** (due to a progressive insulin secretory defect on the background of insulin resistance)

- **Other specific types** of diabetes due to other causes, e.g., genetic defects in β-cell function, genetic defects in insulin action, diseases of the exocrine pancreas (such as cystic fibrosis), and drug- or chemical-induced (such as in the treatment of HIV/AIDS or after organ transplantation)

- **Gestational diabetes mellitus** (GDM) (diabetes diagnosed during pregnancy that is not clearly overt diabetes)

However, in vast majority of cases, diabetes fall into two broad etiopathogenetic categories such as type 1 and type 2 diabetes.

**Type 1 diabetes**

Type 1 diabetes is characterized by an immune-mediated depletion of β-cells that results in lifelong dependence on exogenous administration of insulin. Type 1 diabetes is usually diagnosed in children and young adults, and was previously known as juvenile diabetes. About 5% of people with diabetes have this form of the disease. In type 1 diabetes, the body does not produce insulin, a hormone that is needed to convert sugar, starches and other food into energy needed for daily life (Chiang JL et al., 2014). Administration of insulin in the form of injections is the only option for the treatment of type 1 diabetes.
In general, T1D is characterized by a long pre-clinical phase between genetic susceptibility, active autoimmunity and finally the development of overt diabetes. At the time of diagnosis, islets of Langerhans are infiltrated by immune cells; a process called insulitis (GeptsW, 1965) and at that moment around 70-80% of the total β-cell mass is already lost, making prediction and prevention a high priority. Several studies indicated that induction of insulitis can also be caused by aspecific viral infection (Hyöty et al., 1998; Drescher et al., 2008). Endogenous and exogenous viral dsRNA binds to β-cell pattern recognition receptors (PRRs) like Toll-like receptor 3 (TLR3), Retinoic acid-inducible gene 1 (RIG-I) and Melanoma Differentiation Associated protein 5 (MDA5), leading to activation of transcription factors such as Signal Transducer and Activator of Transcription 1 (STAT1), Nuclear factor-kappa-B (NF-κB) and Interferon regulatory factor 3 (IRF3) (Rasschaert et al., 2005; Dogusan et al., 2008). Activation of these specific pathways results in the release of chemokines and cytokines responsible for the attraction and activation of innate and adaptive immune cells in the islets.

In addition, Major histocompatibility complex (MHC) class I molecules that present β-cell antigens are upregulated, endoplasmic reticulum (ER) stress is generated and β-cells undergo apoptosis (Eizirik et al., 2009). Apoptotic β-cells are removed by antigen presenting cells, such as macrophages and dendritic cells, which present β-cell antigen-derived peptides on their cell surface in association with MHC class II molecules.
Native, infiltrated CD4⁺ T-lymphocytes with T-cell receptors (TCR) recognizing these peptides, will become activated effector T-lymphocytes which will stimulate the maturation of CD8⁺ T-lymphocytes into cytotoxic T-lymphocytes by production of cytokines such as interleukin 2 (IL-2). These cytotoxic T-cells recognize β-cell antigens presented by MHC class I molecules on the β-cell surface resulting in granzyme- and perforin-mediated β-cell death (Thomas et al., 2010).

Effector T-lymphocytes also secrete Interferon γ (IFNγ), leading to activation of macrophages and subsequent release of chemokines, free radicals and the inflammatory cytokines IL-1beta, IFNγ and Tumor necrosis factor α (TNFα). This inflammatory environment further induces increasing amounts of ER stress in β-cells, decreases insulin production and secretion in response to glucose and finally leads to β-cell death. Under these circumstances, β-cells are stimulated to release more chemokines and cytokines, leading to the attraction of even more macrophages and T-lymphocytes (Eizirik et al., 2003; Eizirik et al., 2008).

In summary, the process of insulitis, which started with an innocent aspecific inflammation, turns into a vicious circle characterized by a dialogue between β-cells and immune cells resulting in massive inflammation of the islets, leading to defective insulin production and finally T1D.
Type 2 diabetes

Type 2 DM was first described as a component of metabolic syndrome in 1988. Type 2 DM (formerly known as non-insulin dependent DM) is the most common form of DM characterized by hyperglycemia, insulin resistance, and relative insulin deficiency (ADA, 2009). Type 2 DM is mainly due to the interactions between genetic, environmental and behavioral risk factors. Individuals living with type 2 DM are more vulnerable to various forms of both short- and long-term complications, which often lead to their premature death.

Lifestyle, Genetics, and Medical Conditions

Type 2 DM is due primarily to lifestyle factors and genetics (Ripsin et al., 2009). A number of lifestyle factors are known to be important to the development of type 2 DM. These are physical inactivity, sedentary lifestyle, cigarette smoking and generous consumption of alcohol (Hu et al., 2001). Obesity has been found to contribute to approximately 55% of cases of type 2 DM (CDC, 2004). The increased rate of childhood obesity between the 1960s and 2000s is believed to have led to the increase in type 2 DM in children and adolescents (Barlow, 2007). Environmental toxins may contribute to the recent increases in the rate of type 2 DM. A weak positive correlation has been found between the concentrations in the urine of bisphenol A, a constituent of some plastics, and the incidence of type 2 DM (Lang et al., 2008).
There is a strong inheritable genetic connection in type 2 DM, having relatives (especially first degree) with type 2 DM increases the risks of developing type 2 DM substantially. Concordance among monozygotic twins is close to 100%, and about 25% of those with the disease have a family history of DM (Rother, 2007). Recently, genes discovered to be significantly associated with developing type 2 DM, include TCF7L2, PPARG, FTO, KCNJ11, NOTCH2, WFS1, CDKAL1, IGF2BP2, SLC30A8, JAZF1, and HHEX. KCNJ11 (potassium inwardly rectifying channel, subfamily J, member 11), encodes the islet ATP-sensitive potassium channel Kir6.2, and TCF7L2 (transcription factor 7-like 2) regulates proglucagon gene expression and thus the production of glucagon-like peptide-1 (McCarthy, 2010). Moreover, obesity (which is an independent risk factor for type 2 DM) is strongly inherited (Walley et al., 2006).

Monogenic forms like Maturity-onset diabetes of the young (MODY), constitute up to 5% of cases (Camastra et al., 1999). There are many medical conditions which can potentially give rise to, or exacerbate type 2 DM. These include obesity, hypertension, elevated cholesterol (combined hyperlipidemia), and with the condition often termed metabolic syndrome (it is also known as Syndrome X, Reaven's syndrome) (Alberti et al., 2005). Other causes include Acromegaly, Cushing's syndrome, Thyrotoxicosis, Pheochromocytoma, Chronic pancreatitis, Cancer and certain drugs (Powers, 2008). Additional factors found to increase the risk...
of type 2 DM include aging, high-fat diet, and a less active lifestyle (Jack et al., 2004).

**Pathophysiology**

Type 2 DM is characterized by insulin insensitivity as a result of insulin resistance, declining insulin production, and eventual pancreatic β-cell failure (Kahn, 1994; Robertson, 1995). This leads to a decrease in glucose transport into the liver, muscle cells, and fat cells. There is an increase in the breakdown of fat with hyperglycemia. The involvement of impaired alpha-cell function has recently been recognized in the pathophysiology of type 2 DM (Fujioka, 2007). As a result of alpha-cell dysfunction, glucagon and hepatic glucose levels that rise during fasting are not suppressed with a meal. Given inadequate levels of insulin and increased insulin resistance, hyperglycemia results. Also very important is adipose tissue, as endocrine organ hypothesis (secretion of various adipocytokines, i.e., leptin, TNF-α, resistin, and adiponectin) has been implicated in insulin resistance and possibly β-cell dysfunction (Fujioka, 2007).

A majority of individuals suffering from type 2 DM are obese, with central visceral adiposity. Therefore, the adipose tissue plays a crucial role in the pathogenesis of type 2 DM. Although, the predominant theory used to explain this link is the oral and visceral hypothesis giving a key role in elevated non-esterified fatty acid concentrations, the two new emerging theories are the ectopic fat storage syndrome (deposition of triglycerides in
muscle, liver and pancreatic cells). These two hypotheses constitute the framework for the study of the interplay between insulin resistance and β-cell dysfunction in type 2 DM as well as between our obesogenic environment and DM risk in the next decade (Fujioka, 2007).

**Figure 1: Pathogenesis of type 2 diabetes**

**Other specific types**

- Genetic defects of β-cell function
- Genetic defects of insulin action (e.g. leprechaunism, lipodystrophies)
- Pancreatic disease (e.g. pancreatitis, pancreatectomy, neoplastic disease, cystic fibrosis, haemochromatosis, fibrocalculous pancreateopathy)
Excess endogenous production of hormonal antagonists to insulin (e.g. growth hormone – acromegaly; glucocorticoids – Cushing’s syndrome, glucagon – glucagonoma; catecholamines – phaeochromocytoma; thyroid hormones – thyrotoxicosis)

Drug induced (e.g. corticosteroids, thiazide diuretics, phenytoin)

Viral infections (e.g. congenital rubella, mumps, coxsackie virus b)

Uncommon forms of immune-mediated diabetes

Associated with genetic syndromes (e.g. Down’s syndrome, Turner’s syndrome, Klinefelter’s syndrome, nerve deafness, Freidreich’s ataxia, myotonic dystrophy.

**Gestational diabetes**

Gestational diabetes mellitus (GDM) is defined as ‘carbohydrate intolerance resulting in hyperglycemia of variable severity with onset or first recognition during pregnancy’ (WHO, 1999; Expert, 2000). GDM is defined in this way for women with undiagnosed pre-existing diabetes and for those in whom the first onset of diabetes is seen during pregnancy. To identify early in pregnancy women with previously undiagnosed diabetes, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) (Metzger et al., 2010) recommends assessment of high-risk populations using glycated haemoglobin, fasting or random plasma glucose at first pregnancy.
In the past, GDM has been categorized by severity of glucose impairment, with less severe cases referred to as “impaired glucose tolerance” and more severe cases as GDM. Hyperglycemia is now viewed as a continuum, with higher levels along the completedistribution of glycemia in pregnant women associated with adverseeffects (HAPO Study Cooperative Research Group, 2008). Because of this, the term ‘impaired glucose tolerance’ is no longer used, and all abnormalities of glucose intolerance in pregnancy are now referred to as “gestational diabetes mellitus.” GDM necessarily includes women who first develop diabetes during pregnancy, as well as those with previously undiagnosed type 1 or 2 diabetes mellitus (frank diabetes) recognized for the first time in pregnancy, because it is impossible at the time of first recognition to distinguish clearly between these different diagnoses. GDM complicates approximately 7% of pregnancies (Gabb et al., 1977; ADA, 2004), although this varies with the population characteristics and the diagnostic glucose thresholds used.

**DIAGNOSTIC CRITERIA FOR DIABETES MELLITUS**

For the last decades, the diagnosis of diabetes is based on fasting plasma glucose (FPG) and oral glucose tolerance test (OGTT).

The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus of the American Diabetes Association states that diabetes can be provisionally diagnosed with any one of the four criteria listed below. In the absence of unequivocal hyperglycemia with acute metabolic
decompensation, the diagnosis should be confirmed, on a subsequent day, by any one of the same four criteria.

**Criteria for the diagnosis of diabetes** (American Diabetes Association, 2012)

1. **HbA1C ≥6.5%**. The test should be performed in a recognized laboratory using a method that is National Glycohemoglobin Standardization Program (NGSP) certified and standardized to The Diabetes Control and Complications Trial (DCCT) assay.

   OR

2. **FPG ≥126 mg/dl** (7.0 mmol/l). Fasting is defined as no caloric intake for at least 8 h.

   OR

3. **2-h plasma glucose ≥200 mg/dl** (11.1 mmol/l) during an OGGT. The test should be performed as described by the World Health Organization, using oral glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.

   OR

4. In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dl (11.1 mmol/l).

   **In the absence of unequivocal hyperglycemia, criteria should be confirmed by repeated 1–3 tests.**
HbA1C is a widely used marker of chronic glycemia, reflecting the average blood glucose levels maintained over a 2- to 3-month period of time. The test plays a critical role in the management of the patients with diabetes, since it correlates well with both microvascular and, to a lesser extent, macrovascular complications and is widely used as the standard biomarker for the adequacy of glycemic management. Prior Expert Committees have not recommended use of the HbA1C for diagnosis of diabetes, in part due to lack of standardization of the assay. However, HbA1C assays are now highly standardized so that their results can be uniformly applied both temporarily and across the population. In their recent report, an International Expert Committee, after an extensive review of both established and emerging epidemiological evidence, recommended the use of the HbA1C test to diagnose diabetes, with a threshold of ≥6.5%, and ADA affirms this decision. The diagnostic HbA1C cut point of 6.5% is associated with an inflection point for retinopathy prevalence, as are the diagnostic thresholds for FPG and 2-h PG (3). The diagnostic test should be performed using a method that is certified by the NGSP and standardized or traceable to the Diabetes Control and Complications Trial reference assay. Point-of-care HbA1C assays are not sufficiently accurate at this time to use for diagnostic purposes (Gillett, 2009).

**Complications of diabetes mellitus**

Diabetes mellitus is a very common disease, characterized by an asymptomatic phase between the actual onset of diabetic hyperglycemia and clinical diagnosis. This phase has been estimated to last at least 4–7 years, and in 30–50% cases of type 2 diabetic patients remained undiagnosed (Harris et
al., 1992). This leads to the development of microvascular complications (diabetic nephropathy, neuropathy, and retinopathy) and macrovascular complications (coronary artery disease, peripheral arterial disease, and stroke) of diabetes, which remain the chief problems in diabetic care, and which cause a lack of fitness to work, disability, and premature death (Spijkerman et al., 2003; Piechowski-Jozwiak et al., 2005).

**Figure 2: Complications of diabetes mellitus**

**Microvascular complications**
**Diabetic retinopathy**

Diabetic retinopathy may be the most common microvascular complication of diabetes. It is responsible for ~ 10,000 new cases of blindness every year in the United States alone (Fong et al., 2004). The risk of developing diabetic retinopathy or other microvascular complications of diabetes depends on both the duration and the severity of hyperglycemia. Development of diabetic retinopathy in patients with type 2 diabetes was found to be related to both severity of hyperglycemia and presence of hypertension in the U.K. Prospective Diabetes Study (UKPDS), and most patients with type 1 diabetes develop evidence of retinopathy within 20 years of diagnosis (UKPDS, 1998; Keenan et al., 2007). Retinopathy may begin to develop as early as 7 years before the diagnosis of diabetes in patients with type 2 diabetes (Fong et al., 2004). There are several proposed pathological mechanisms by which diabetes may lead to development of retinopathy.

Cells are also thought to be injured by glycoproteins. High glucose concentrations can promote the non-enzymatic formation of advanced glycosylated end products (AGEs). In animal models, these substances have also been associated with formation of microaneurysms and pericyte loss (Fong et al., 2004).

Aldose reductase may participate in the development of diabetes retinopathy. Aldose reductase is the initial enzyme in the intracellular polyol pathway. This pathway involves the conversion of glucose into glucose
alcohol (sorbitol). High glucose levels increase the flux of sugar molecules through the polyol pathway, which causes sorbitol accumulation in cells. Osmotic stress from sorbitol accumulation has been postulated as an underlying mechanism in the development of diabetic microvascular complications, including diabetic retinopathy. In animal models, sugar alcohol accumulation has been linked to microaneurysm formation, thickening of basement membranes, and loss of pericytes (Gabbay, 1975; Gabbay, 2004).

Oxidative stress may also play an important role in cellular injury from hyperglycemia. High glucose levels can stimulate free radical production and reactive oxygen species formation. Animal studies have suggested that treatment with antioxidants, such as vitamin E, may attenuate vascular dysfunction associated with diabetes, but treatment with antioxidants has not yet been shown to alter the development or progression of retinopathy or other microvascular complications of diabetes (Kunisaki et al., 1995).

**Diabetic nephropathy**

Diabetic nephropathy is the leading cause of renal failure. It is defined by proteinuria > 500 mg in 24 hours in the setting of diabetes, but this is preceded by lower degrees of proteinuria, or “microalbuminuria.” Microalbuminuria is defined as albumin excretion of 30-299 mg/24 hours. Without intervention, diabetic patients with microalbuminuria typically
progress to proteinuria and overt diabetic nephropathy. This progression occurs in both type 1 and type 2 diabetes (Chaturvedi et al., 2001).

As many as 7% of patients with type 2 diabetes may already have microalbuminuria at the time they are diagnosed with diabetes. In the European Diabetes Prospective Complications Study (EDPCS), the cumulative incidence of microalbuminuria in patients with type 1 diabetes was \( \sim 12\% \) during a period of 7 years. In the UKPDS, the incidence of microalbuminuria was 2% per year in patients with type 2 diabetes, and the 10-year prevalence after diagnosis was 25% (Adler et al., 2003).

The pathological changes to the kidney include increased glomerular basement membrane thickness, microaneurysm formation, mesangial nodule formation (Kimmelsteil-Wilson bodies), and other changes. The underlying mechanism of injury may also involve some or all of the same mechanisms as diabetic retinopathy.

In addition to its being the earliest manifestation of nephropathy, albuminuria is a marker of increased cardiovascular morbidity and mortality in patients with either type 1 or type 2 diabetes. Thus, the finding of microalbuminuria is an indication to screen the possible vascular disease and aggressive intervention to reduce all cardiovascular risk factors (e.g., lowering of LDL cholesterol, antihypertensive therapy, cessation of smoking, institution of exercise, etc.). In addition, there is some preliminary evidence to
suggest that lowering of cholesterol may also reduce the level of proteinuria (Gross et al., 2005).

**Diabetic neuropathy**

Diabetic neuropathy is recognized by the American Diabetes Association (ADA) as the presence of symptoms and/or signs of peripheral nerve dysfunction in individuals with diabetes after the exclusion of other causes (ADA, 2007). As with other microvascular complications, the risk of developing diabetic neuropathy is proportional to both the magnitude and duration of hyperglycemia, and some individuals may possess genetic attributes that affect their predisposition to developing such complications.

The precise nature of injury to the peripheral nerves from hyperglycemia is not known, but likely is related to mechanisms such as polyol accumulation, injury from AGEs, and oxidative stress. Peripheral neuropathy in diabetes may manifest in several forms, including sensory, focal/multifocal, and autonomic neuropathies. More than 80% of amputations occur after foot ulceration or injury, which can result from diabetic neuropathy (Boulton et al., 2005). Because of the considerable morbidity and mortality that can result from diabetic neuropathy, it is important for clinicians to understand its manifestations, prevention, and treatment.

Chronic sensorimotor distal symmetric polyneuropathy is the most common form of neuropathy in diabetes. Typically, patients experience burning, tingling, and “electrical” pain, but sometimes they may experience
simple numbness. In patients who experience pain, it may be worse at night. Patients with simple numbness can present with painless foot ulceration, so it is important to realize that lack of symptoms does not rule out the presence of neuropathy (Boulton et al., 2005).

There is no specific treatment of diabetic neuropathy, although many drugs are available to treat its symptoms. The primary goal of therapy is to control symptoms and prevent worsening of neuropathy through improved glycemic control. Some studies have suggested that control of hyperglycemia and avoidance of glycemic excursions may improve symptoms of peripheral neuropathy (Fowler, 2008).

**Macrovascular complications**

**Cardiovascular disorders**

The central pathological mechanism in macrovascular disease is the process of atherosclerosis, which leads to narrowing of arterial walls throughout the body. Atherosclerosis is thought to result from chronic inflammation and injury to the arterial wall in the peripheral or coronary vascular system. In response to endothelial injury and inflammation, oxidized lipids from LDL particles accumulate in the endothelial wall of arteries. Angiotensin II may promote the oxidation of such particles. Monocytes can infiltrate the arterial wall and differentiate into macrophages, which accumulate oxidized lipids to form foam cells. Once formed, foam cells stimulate macrophage proliferation and attraction of T-lymphocytes, which in
turn, induce smooth muscle proliferation in the arterial walls and collagen accumulation. The net result of the above processes is the formation of a lipid-rich atherosclerotic lesion with a fibrous cap. Rupture of the atherosclerotic lesion leads to acute vascular infarction (Boyle, 2007).

In addition to atheroma formation, there is strong evidence of increased platelet adhesion and hypercoagulability in type 2 diabetes. An atheroma is an accumulation of degenerative material in the inner layer of artery walls. Impaired nitric oxide generation and increased free radical formation in platelets, as well as altered calcium regulation, may promote platelet aggregation (Fowler, 2008). Elevated levels of plasminogen activator inhibitor type 1 may also impair fibrinolysis in individuals with diabetes. The combination of increased coagulability and impaired fibrinolysis may increase the risk of vascular occlusion and cardiovascular events in type 2 diabetes (Beckman et al., 2002).

Diabetes increases the risk that an individual will develop cardiovascular disease (CVD). Although the precise mechanisms through which diabetes increases the likelihood of atherosclerotic plaque formation are not completely defined, the association between the two is profound. CVD is the primary cause of death in people with either type 1 or type 2 diabetes (Laing et al., 2003; Paterson et al., 2007). In fact, CVD accounts for the greatest component of health care expenditures in people with diabetes (Hogan et al., 2002).
Type 2 diabetes typically occurs in the onset of metabolic syndrome, which mainly includes abdominal obesity, hypertension, hyperlipidemia, and increased coagulability. These factors can also act to promote CVD. Even in this setting of multiple risk factors, type 2 diabetes acts as an independent risk factor for the development of ischemic disease, stroke, and death (Almdal et al., 2004). Among the individuals with type 2 diabetes, women may be at higher risk for coronary heart disease than men. The presence of microvascular disease is also a predictor of coronary heart events (Avogaro et al., 2007).

The increased risk of CVD has led to more aggressive treatment of pathological conditions to achieve primary or secondary prevention of coronary heart disease before it occurs. Studies in type 1 diabetes have shown that intensive diabetes control is associated with a lower resting heart rate and that patients with higher degrees of hyperglycemia tend to have a higher heart rate, which is associated with higher risk of CVD. Even more conclusively, the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study demonstrated that during 17 years of prospective analysis, intensive treatment of type 1 diabetes, including lower HbA1C, is associated with a 42% risk reduction in all cardiovascular events and a 57% reduction in the risk of nonfatal MI, stroke, or death from CVD (Nathan et al., 2005).

Overview of the pathogenesis of type 2 diabetes
Any rise in glycemia is the net result of glucose influx exceeding glucose outflow from the plasma compartment. In the fasting state, hyperglycemia is directly related to increased hepatic glucose production. In the postprandial state, further glucose excursions result from the combination of insufficient suppression of this glucose output and defective insulin stimulation of glucose disposal in target tissues, mainly skeletal muscle. Once the renal tubular transport for glucose is exceeded the threshold value, glycosuria curbs, though does not prevent, further hyperglycemia. Abnormal islet cell function is a key feature of type 2 diabetes. In early disease stages, insulin production is normal or increased in absolute terms, but disproportionately low for the degree of insulin sensitivity, which is typically reduced. However, insulin kinetics, such as the ability of the pancreatic β-cell to release adequate hormone in phase with rising glycemia, are profoundly compromised. This functional islet incompetence is the main quantitative determinant of hyperglycemia (Ferrannini et al., 2005) and progresses over time. In addition, in type 2 diabetes, pancreatic alpha cells hypersecrete glucagon, further promoting hepatic glucose production (Nauck, 2011). Importantly, islet dysfunction is not necessarily irreversible. Enhancing insulin action relieves β-cell secretory burden, and any intervention that improves glycemia—from energy restriction to, most strikingly, bariatric surgery—can ameliorate β-cell dysfunction to an extent (Ferrannini, 2010).

More recently recognized abnormalities in the incretin system (represented by the gut hormones, glucagon-like peptide 1 [GLP-1] and
glucose-dependent insulino-tropic peptide (GIP) are also found in type 2 diabetes, but it remains unclear whether these constitute primary or secondary defects (Nauck, 2009). Current theories of T2DM include a defect in insulin-mediated glucose uptake in muscle, a dysfunction of the pancreatic β-cells, a disruption of secretory function of adipocytes, and an impaired insulin action in liver. This results in both glucose overproduction and underutilisation. Moreover, an increased delivery of fatty acids to the liver favours their oxidation, which contributes to increased gluconeogenesis, whereas the absolute overabundance of lipids promotes hepatosteatosis (Groop and Ferrannini, 1993). In addition, studies are ongoing on the role of mitochondrial dysfunction in the development of insulin resistance and etiology of type 2DM.

**Skeletal muscle**

Skeletal muscle is a key metabolic tissue, which is responsible for more than 80% of total glucose disposal under normal insulin-stimulated conditions (Taube et al., 2009). Plasma insulin concentration restrains lipolysis in adipocytes and stimulates glucose uptake in skeletal muscle. During fasting, muscle glucose uptake is low and the plasma FFA concentration is elevated (Abdul-Ghani and DeFronzo, 2010). Under normal conditions, skeletal muscle contains the majority of the glycogen stores and a small amount of intramyocellular TG. Skeletal muscle has a capacity to utilize either lipids or carbohydrates as a source of energy and effectively transit between these fuel sources. Small amounts of intracellular lipids are
important energy sources for skeletal muscle during low glucose supply (Kelley et al., 2002).

Patients with T2DM are characterized by a decreased fat oxidative capacity and high levels of circulating free fatty acids. The latter is known to cause insulin resistance, particularly in skeletal muscle, by reducing insulin-stimulated glucose uptake, most likely via accumulation of lipid inside the muscle cell. Increased lipolysis in peripheral adipose tissue leads to lipid oversupply and storage of available FFAs in muscle cells when they are no longer isaccomplished by adipose tissue (Taube et al., 2009). Increased intramyocellular lipid (IMCL) has been linked to obesity and decreased whole body insulinsensitivity (Kotronen et al., 2008) and skeletal muscle triglyceride content is insignificantly increased in T2DM (He and Kelley, 2004). Furthermore, it is hypothesized that insulin resistance in skeletal muscle decreases non-oxidative storage of ingested carbohydrates, which are then converted into liver for hepatic de novo lipogenesis resulting in peripheral hypertriglycerideremia (Jornayvaz et al., 2010).

Increased intramyocellular lipids have also been reported to be associated with impaired insulin-induced glucose metabolism (Hirabara et al., 2010). There is also evidence that skeletal muscle mitochondrial dysfunction is a major cause of insulin resistance and T2DM rather than triglyceride content per se (Schrauwen-Hinderling et al., 2007), which means that increased intramyocellular lipid is not independently responsible for the development of T2DM. Intramyocellular lipid accumulation has also been associated with
decreased peripheral insulin sensitivity in healthy individuals (Salgin et al., 2009), suggesting that increased intramyocellular lipids may be an early step in the development of T2DM.

Liver

The liver is a central organ in lipogenesis, gluconeogenesis and cholesterol metabolism. Under fasting conditions, hepatic fatty acids are derived mainly from adipose tissue lipolysis and less than 5% of intrahepatocellular TGs originate from de novo lipogenesis in the hepatocytes (Barrows and Parks, 2006). Very low density lipoprotein (VLDL) FFA spillover from peripheral tissue lipolysis is also a source of intrahepatocellular TGs (Donnelly et al., 2005; Goldberg and Ginsberg, 2006). Obesity and insulin resistance are regarded to be key mechanisms leading to accumulation of TGs in the liver. It is postulated that fatty liver is a result rather than a cause of peripheral insulin resistance in obesity (Liu et al., 2010). The associations between visceral fat, insulin resistance and hepatic steatosis have been widely reported. Noteworthy, diabetic patients have been reported to be insulin resistant at the level of adipose tissue, liver, and muscle and hepatic insulin resistance plays a role in whole body insulin resistance (Magkos et al., 2012).
Visceral obesity in rodents is associated with alterations in insulin signaling, insulin resistance, decreased peroxisome proliferator-activated receptor alpha expression (PPARα) and hepatic steatosis. These alterations may lead to hepatic oxidative stress, liver injury, cell apoptosis, and collagen deposition. However, the two major mechanisms leading to liver fat accumulation: adipose tissue lipolysis and peripheral hyperinsulinemia (Angulo, 2002). Furthermore, impaired fatty acid oxidation and increased hepatic de novo lipogenesis play a key role in the pathogenesis of liver fat accumulation.

In obesity and insulin resistance, hypertrophied intraabdominal adipocytes are resistant to the antilipolytic effect of insulin. This leads to increased lipolysis in the peripheral adipose tissue and excess FFA flux to the liver. In hepatocytes, hyperinsulinemia increases de novo synthesis of fatty acids. Increased fatty acid uptake and lipogenesis by hepatocytes together lead to mitochondrial β-oxidation overload and inadequate compensatory fat oxidation. There is evidence that free fatty acids themselves may not be sufficient to induce fatty liver disease (Parekh and Anania, 2007). The pathogenesis of diabetes in liver is widely recognized as a two-hit model. The “first hit” consists of metabolic disturbances that increase the inflow of FFAs into the liver and de novo lipogenesis, leading to hepatic steatosis. Increased levels of intrahepatic fatty acids are a source of oxidative stress. The “second hit” includes oxidative stress from
mitochondria and cytochrome P-450 system, which is characterized by excessive production of reactive oxygen species (ROS), decreased hepatic ATP production, and increased expression and induction of inflammatory cytokines. These factors may trigger necroinflammation leading from steatosis to the progression of steatohepatitis (Rolo et al., 2012).

**Adipose tissue**

Under normal physiologic conditions, insulin controls the balance between postprandial fatty acid storage as triglycerides and their release into the circulation during the fasting state. Adipose tissue is extremely sensitive to insulin concentrations, inhibiting lipolysis at insulin concentrations that are much lower than those needed to inhibit hepatic glucose production or stimulate muscle glucose uptake (Choi et al., 2010).

In obesity and T2DM, there is marked adipocyte resistance to the antilipolytic effect of insulin and plasma FFA concentrations are typically elevated. Under the metabolic stress induced by chronic overfeeding, adipocytes undergo pathologic enlargement (hypertrophy) and fail to adequately proliferate and differentiate, and the endoplasmic reticulum (ER) triggers the unfolded protein response (UPR) as a protective mechanism. The UPR leads to inflammatory responses within the fat cell mediated through several signaling pathways, such as c-Jun N-terminal kinase (JNK), inhibitor
κB kinase (IKK)/nuclear factor-κB (NF-κB), cyclicAMP-responsive element-binding protein H (CREBH, promoting the secretion of acute-phase proteins such as C-reactive protein), and production of reactive oxygen species (Johnson et al., 2012). Weight loss can reverse these abnormalities. Adipocytes secrete a host of cytokines in response to metabolic stress capable of causing local paracrine effects on macrophages and more distal endocrine effects such as muscle and liver insulin resistance (i.e., tumor necrosis factor-α [TNF-α], interleukin-1, -6, and -8, resistin, monocyte chemoattractant protein-1 [MCP-1], plasminogen activator inhibitor-1, visfatin, angiotensinogen, retinol-binding protein-4, serum amyloid A, transforming growth factor-β, and others) (Roberts et al., 2013).

Adipose tissue is formed not only by mature adipocytes, but also by stromal preadipocytes, immune cells, extracellular matrix, and the vascular endothelium. Macrophage infiltration promotes adipose tissue insulin resistance, excessive release of FFA, and ectopic fat deposition. During nutritional excess, hypertrophic adipocytes develop a gene expression pattern that closely resembles that of macrophages (although macrophages derive from bone marrow stem cells, a different cellular lineage), and produce cytokines of the kind described in foam cells, the fat-loaded activated macrophages that are found in arterial plaques (Rosen and MacDougald, 2006). It has also been recently proposed that upregulation of the extracellular matrix and “adipose tissue fibrosis” in obesity, specifically upregulation of collagen VI, restricts adipocyte growth and contributes to metabolic
dysfunction, whereas its absence allows unrestricted adipocyte expansion and is associated with metabolic improvement (Sun et al., 2011).

**Pancreatic β-cell**

Apoptosis is an active process of cellular self-destruction that is regulated by the extrinsic and intrinsic signals occurring in normal development. Apoptosis plays an important role in the development of diabetes and mediates β-cell destruction in type 1 diabetes mellitus (Kurrer et al., 1997) and a reduction in β-cell mass in type 2 diabetes mellitus (Pick et al., 1998). Several mechanisms have been shown to induce apoptosis in β-cells which include endoplasmic reticulum stress (Harding and Ron, 2002), chronic hyperglycemia (Donath and Halban, 2004), chronic hyperlipidemia (Poitout and Robertson, 2002), oxidative stress (Kaneto et al. 2006), and inflammatory cytokines (Donath et al., 2003). Decreased insulin receptor substrate (IRS2) expression may also lead to spontaneous β-cell apoptosis (Hennige et al., 2003).

Several mechanisms relevant to pathogenesis of T2DM may increase IRS2 serine/threonine phosphorylation (Werner et al., 2004) with resultant IRS2 ubiquitination and proteosomal degradation, defects in insulin signaling and insulin secretion may be coupled. When β-cell function is viewed in the context of reduced insulin sensitivity, considerable data support the early failure of insulin secretion in T2DM pathogenesis (Kahn, 2003). Animal studies also support this concept. However, the signals that cause
normal β-cell compensation and hyperinsulinemia, the mechanisms of this compensation, the point in the pathogenesis of T2DM where this compensatory mechanism fails, and the etiology of this failure all remain unclear.

**Free fatty acid, Oxidative stress and Insulin resistance**

Randle demonstrated that impairment of glucose metabolism by fatty acid (or ketone body) oxidation was mediated by a short-term inhibition of several glycolytic steps, namely glucose transport and phosphorylation, 6-phosphofructo-1-kinase (PFK-1), and Pyruvate Dehydrogenase (PDH) (Randle et al., 1963). The extent of inhibition is graded and increases along the glycolytic pathway, being most severe at the level of PDH and less severe at the level of glucose uptake and Phosphofructokinase. This sequence occurs because the initial event, triggered by fatty acid oxidation, is an increase in the mitochondrial ratios of [acetyl-CoA]/[CoA] and [NADH]/[NAD], both of which inhibit PDH activity. It has been proposed that these changes lead to an accumulation of cytosolic citrate, which in turn inhibits PFK-1, followed by an increase in glucose 6-phosphate, which eventually inhibits hexokinase (Hue and Taegtmeyer, 2009).

A causative role for elevated free fatty acid (FFA) levels in the development of microvascular complications remains to be established. However, increased levels of FFAs are positively correlated with both insulin resistance (McGarry, 2002) and the deterioration of β-cell function.
in the context of concomitant hyperglycemia (Harmon, 2001). These latter effects may result from oxidative stress.

There is evidence that oxidative stress, defined as a persistent imbalance between the production of highly reactive molecular species and antioxidant defenses, leads to tissue damage. Oxidative stress results from increased content of reactive oxygen species (ROS) and/or reactive nitrogen species (RNS). Examples of ROS include charged species such as superoxide and the hydroxyl radical and uncharged species such as hydrogen peroxide (Rosen et al., 2001). There are data indicating that ROS formation is a direct consequence of hyperglycemia. Studies that are more recent have suggested that increased FFA levels may also result in ROS formation (Brownlee, 2001).

Because of their ability to directly oxidize and damage DNA, protein, and lipid, ROS are believed to play a key direct role in the pathogenesis of late diabetic complications. In addition to their ability to directly inflict macromolecular damage, ROS can function as signaling molecules to activate a number of cellular stress-sensitive pathways that cause cellular damage, and are ultimately responsible for the late complications of diabetes. Furthermore, these same pathways are linked to insulin resistance and decreased insulin secretion. ROS and oxidative stress induced by elevations in glucose and possibly FFA levels play a key role in causing insulin resistance and β-cell dysfunction by their ability to activate stress-sensitive signaling pathways (Nishikawa et al., 2000).
Insulin resistance causes hyperglycemia and lack of insulin signaling decreases transport of glucose into muscle and fat, while increasing glucose production by the liver (DeFronzo et al., 1989). Hyperglycemia-induced increases in the production of $\mathrm{O}_2^-$ by the mitochondrial ETC in endothelial cells have been implicated in glucose-mediated vascular damage (Brownlee, 2001). Normalizing mitochondrial ROS levels by an inhibitor of electron transport complex II, by an uncoupler of oxidative phosphorylation, by overexpression of UCP-1 or SOD2 each prevented glucose-induced activation of protein kinase C (PKC), formation of advanced glycation end products (AGE), and activation of the polyol pathway, which results in sorbitol accumulation and nuclear factor $\kappa\mathrm{B}$ activation all of which have been implicated in hyperglycemia-induced vascular dysfunction, including atherosclerosis. Activation of nuclear factor $\kappa\mathrm{B}$ induces expression of vascular cell adhesion molecule-1 and monocyte chemoattractant protein-1 in aortic endothelial cells stimulating atherogenesis (Verma et al., 2002).

Hyperglycemia-induced increase in mitochondrial $\mathrm{O}_2^-$ production also decreases glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity and increases hexosamine pathway activity in aortic endothelial cells (Du et al., 2000). Activation of the hexosamine pathway causes increased glycosylation and subsequent transactivation of transcription factor Sp1, resulting in increased expression of Sp1-dependent genes such as transforming growth factor-1 and plasminogen activator inhibitor-1. Elevated plasma levels of
plasminogen activator inhibitor-1 are strongly associated with increased risk of ischemic heart disease whereas transforming growth factor-1 plays a key role in early atherosclerosis and restenosis (Kohler and Grant, 2000).

Further, it was shown that the activation of the major pathways of hyperglycemic damage in endothelial cells induced by enhanced mitochondrial O$_2^-$ production is mediated via inhibition of glycolytic enzyme, GAPDH. The GAPDH inhibition is caused by poly(ADP-ribosyl)ation by poly(ADP ribose) polymerase, which is activated by nuclear DNA strand breaks produced by mitochondrial O$_2^-$ overproduction. Inhibition of GAPDH increases the entry of upstream glycolytic metabolites into pathways of glucose overuse, including increased flux through AGE and PKC glucotoxic pathways (Reusch, 2003).
Endoplasmic Reticulum Stress Links Obesity, Insulin Action, and Type 2 Diabetes

Over the past decade, it has become clear that obesity is associated with the activation of cellular stress signaling and inflammatory pathways (Uysalet al., 1997; Hirosumi et al., 2002). However, the origin of this stress is not known. A key player in the cellular stress response is the ER, a membranous network that functions in the synthesis and processing of secretory and membrane proteins. Certain pathological stress conditions disrupt ER homeostasis and lead to accumulation of unfolded or misfolded proteins in the ER lumen (Hampton, 2000; Harding et al., 2002). To cope with this stress, cells activate a signal transduction system linking the ER lumen with the cytoplasm and nucleus, called the unfolded protein response (UPR). Among the conditions that trigger ER stress are glucose or nutrient deprivation, viral infections, lipids, increased synthesis of secretory proteins, and expression of mutant or misfolded proteins (Ma and Hendershot, 2001; Kaufman et al., 2002).

Several of these conditions occur in obesity. Specifically, obesity increases the demand on the synthetic machinery of the cells in many secretory organ systems. Obesity is also associated with mechanical stress, excess lipid accumulation, abnormalities in intracellular energy fluxes, and nutrient availability. In light of these observations, obesity may be a chronic stimulus...
for ER stress in peripheral tissues and that perhaps ER stress is a core mechanism involved in triggering insulin resistance and type 2 diabetes.

**AMPK – metabolic target for diabetes**

The adenosine monophosphate-activated protein kinase (AMPK) is an important regulatory protein for cellular energy balance and is considered as a master switch of glucose and lipid metabolism in various organs, especially in skeletal muscle and liver. The AMPK complex combines two regulatory (β, 30 kDa; γ, 38-63 kDa) and a catalytic subunit (α, 63 kDa). These subunits are encoded by different genes and several isoforms of each have been discovered: α1, α2, β1, β2, γ1, γ2 and γ3 (Gruzman et al., 2009). Theoretically, there are 12 possible heterotrimeric combinations of AMPK.

**Metabolic Functions of AMPK**

AMPK phosphorylates serine moieties in target proteins. It mostly interacts with a serine moiety within a 9-amino acid motif. In human, this motif is Φ-Ψ-XX-S/T-X-X-Φ, where Φ, Ψ and X denote hydrophobic, basic or any other amino acid, respectively (Wu et al., 2013). Many of these target proteins regulate key metabolic functions, such as glucose uptake, glycolysis, fatty acid oxidation, cholesterol synthesis, glycogen synthesis, gluconeogenesis, protein synthesis or lipolysis. These functions inhibit anabolic processes and conserve ATP, on one hand, and stimulate catabolic pathways to produce ATP, on the other (Hardie, 2007)

*Role of AMPK in Skeletal muscle*
The activation of muscle AMPK by exogenous compounds or by contraction recruits GLUT-4 to the plasma membrane and augments the rate of glucose transport in a non-insulin-dependent manner. AMPK-induced translocation of GLUT-4-containing vesicles to the plasma membrane is preceded by the phosphorylation of the protein AS-160 at Thr642. This phosphorylated form of AS-160 releases the vesicle from intracellular storages and allows their recruitment to the plasma membrane (Miinea et al., 2005). In addition, AMPK upregulates the expression of genes encoding GLUT-4 and hexokinase II and stimulates glycogen synthesis in muscles by allosteric activation of glucose-6-phosphatase-induced activity. Various studies link the glucose transport stimulatory effect of AMPK in skeletal muscles to the activation of ERK1/2, p38-MAPK, Pyk2, PLD, αPKC and Grb2. In addition to the GLUT-4 translocation, AMPK also exerts its anabolic function in skeletal muscles by activating two major citric acid cycle enzymes: citrate synthase and succinate dehydrogenase (Winder et al., 2000; Winder, 2008).

AMPK also stimulates glycolysis in cardiomyocytes (and hepatocytes) by activating 6-phosphofructo-2-kinase (PFK2). AMPK (predominantly complexes with the α2 isoform) has a cardioprotective role in augmenting glucose transport and glycolysis in ischemic hearts (Russell et al., 2004). Increased myocardial ischemia injury due to enhanced post ischemic myocardial apoptosis, extended infarct size and worsened cardiac functional recovery were inflicted in mice bearing a dominant negative AMPKα2 in their cardiomyocytes. In non-insulin-sensitive cells that do not express GLUT-4,
AMPK increases glucose uptake possibly by activating the ubiquitous GLUT-1 that resides in the plasma membrane (Barnes et al., 2002).

**Role of AMPK in Adipose tissue**

The activation of AMPK in fat tissues leads to decreased lipogenic flux, massive fatty acid oxidation and decreased triglyceride synthesis. Fasting, physical exercise or treatment with β-adrenergic agonists activates AMPK via a cAMP-dependent mechanism. The α1 catalytic subunit is the predominant isoform expressed in adipocytes and is critical for the major effects of the AMPK complex (Daval et al., 2006). Not only that AMPK activation in adipocytes just marginally increases glucose uptake, but active AMPK antagonizes the augmenting effects of insulin on GLUT-4-mediated glucose uptake. The mechanism of this phenomenon is not clear, but these findings agree with the view that unlike skeletal muscles, glucose in adipocytes is predominantly utilized anabolically for lipid storage (Salt et al., 2000).

AMPK also regulates lipolysis in adipocytes by inactivating hormone-sensitive lipase (HSL) by a targeted serine phosphorylation. Normally, receptor-coupled adenylate cyclase increases lipolysis via cAMP-dependent protein kinase-A1, which activates HSL. Interestingly, exposure of adipocytes to AICAR blocks lipolysis, which is induced by this mechanism (Sullivan et al., 1994). Both basal and isoproterenol-stimulated lipolysis were elevated and the antilipolytic effect of AICAR was lost in adipocytes from AMPKα1 knockout mice. It appears that
AMPK prevents recycling and release of fatty acids from triglycerides, a process which consumes ATP. AMPKα2 has a direct or indirect role in adipose tissue function since its total deletion in mice resulted in an excessive weight gain upon a high-fat diet, but did not entail glucose intolerance (Villena et al., 2004).

**Role of AMPK in Liver**

The main function of AMPK in the liver is to augment fatty acid oxidation and to prevent cholesterol and triglycerides biosynthesis. Liver-specific AMPKα2 deletion in mice enhances hepatic lipogenesis, increases plasma triglyceride levels and hepatic glucose production. Conversely, overexpression of AMPKα2 in hepatocytes decreases plasma triglyceride level (Foretz et al., 2005). AMPK also reduces mRNA content of the sterol regulatory element binding protein-1 (SREBP-1). Over-expression of this factor has been associated with the increased prevalence of dyslipidemia in type 2 diabetes. Activation of AMPK also reduces the cellular content of the mRNA of the carbohydrate responsive element-binding protein (ChREBP). This factor, otherwise, upregulates lipogenesis and therefore plays a key role in inducing of hyperlipidemia in type 2 diabetes patients (Ben et al., 2008).

AMPK also phosphorylates and deactivates Acetyl CoA carboxylase (ACC), an enzyme that exists as two isoforms: ACC1 (cytoplasmic) and ACC2 (predominantly mitochondrial). The inhibition of the former reduces fatty acid synthesis in cells. Malonyl-CoA, the product
of ACC2, is a potent blocker of carnitine palmitoyltransferase-1 (CPT1), which transports long chain fatty acids to mitochondria. When ACC2 is inhibited, the flux of these fatty acids to mitochondria and their oxidation is increased. In addition, AMPK directly stimulates free fatty acid uptake to cells by translocating the fatty acid translocase CD38 to the plasma membrane (Kim, 1997).

**Role of AMPK in β-cells**

Low glucose levels activate AMPK in β-cells. Over expression of wild type AMPK or constitutively active AMPK, or its pharmacological activation attenuate glucose-induced insulin secretion, whereas the expression of a dominant-negative AMPK in cultured β-cells increases it. It has been suggested that the biguanide metformin affects insulin secretion in β-cells by activating AMPK (Leclerc et al., 2004).

Recent studies on the role of AMPK in the regulation of insulin secretion in β-cells have associated it with the mammalian target of rapamycin (mTOR) pathway, energy availability, proteinsynthesis, cell growth and apoptosis. Collectively, these results demonstrate the complexity of AMPK function in regulating insulin secretion in β-cells and the need for thorough investigations of the complexity of such direct and/or indirect interactions.
Figure 4: Role of AMPK in glucose, lipid and protein metabolism

Metabolic Functions of PPARγ

The peroxisome proliferator-activated receptors (PPARs) form a subfamily of the nuclear receptor superfamily. Three isoforms, encoded by separate genes, have been identified: PPARα, PPARβ and PPARγ. The PPARs are ligand-dependent transcription factors that regulate target gene expression by binding to specific peroxisome proliferator response elements (PPREs) in enhancer sites of regulated genes. Each receptor binds to its PPRE as a heterodimer with a retinoid X receptor (RXR). Upon binding an agonist, the conformation of a PPAR is altered and stabilized such that a binding cleft is created and recruitment of transcriptional coactivators occurs. The result is an increase in gene transcription.
PPAR$\gamma$ is necessary and sufficient to differentiate adipocytes. It was first shown to interact directly with the cis element that regulates adipocyte-specific expression of the fatty acid-binding protein aP2. Introduction of PPAR$\gamma$ into fibroblasts in the presence of weak PPAR ligands induce differentiation of the cells into adipocytes (Tontonoz et al., 1994). Earlier reports suggest that PPAR$\gamma$ heterozygous null mice had reduced amounts of adipose tissue (Rosen et al., 1999). Barak et al. described a homozygous null mouse that exhibited extreme lipodystrophy. PPAR$\gamma$ dominant-negative mutants have been generated. When expressed in 3T3-L1 cells, such mutants inhibited their differentiation into adipocytes. In adipocytes, PPAR$\gamma$ regulates the expression of numerous genes involved in lipid metabolism, including aP2, PEPCK, acyl-CoA synthase and LPL (Schoonjans et al., 1996; Barak et al., 1999).

PPAR$\gamma$ has also been shown to control expression of FATP-1 and CD36, both involved in lipid uptake into adipocytes. These genes have been shown to possess PPREs within their regulatory regions. PPAR$\gamma$ also regulates genes that control cellular energy homeostasis. It has been shown to increase expression of the mitochondrial uncoupling proteins, UCP-1, UCP-2, and UCP-3 in vitro and in vivo. The physiological outcome of these alterations are not yet understood. In contrast to its positive action on the UCPs, PPAR$\gamma$ downregulates leptin, a secreted, adipocyte-selective protein that inhibits feeding and augments catabolic lipid metabolism (Kallen and Lazar, 1996). This receptor activity might explain the increased caloric uptake and storage noted in vivo upon treatment with PPAR$\gamma$ agonists.
Management and Treatment of diabetes mellitus

Management of patients with Diabetes mellitus demands a comprehensive approach which includes diabetes education, an emphasis on lifestyle modification, achievement of good glycemic control, minimization of cardiovascular risk, and avoidance of drugs that can aggravate glucose or lipid metabolism, and screening for diabetes complications.

The success of any treatment is measured by its efficacy for prolonging life, and at the same time allowing them to carry out their usual activities. The treatment of diabetes can be achieved either by non-pharmacological or pharmacological treatment:

Non-pharmacological treatment

Diet

Diet plays a significant role in controlling the diabetes. Dietary management is considered as one of the most important factors in the attainment and maintenance of good metabolic control. Diabetic diet may be used alone or else in combination with insulin doses or with oral hypoglycemic drugs. The diet plan for a diabetic is based on height, weight, age, sex, physical activity and nature of diabetes. The dietitian has to consider complications such as high blood pressure and high cholesterol levels while planning diet. The main objective of diabetic diet is to maintain ideal body
weight, by providing adequate nutrition along with normal blood sugar levels in blood. Landmark clinical trials of lifestyle changes in subjects with prediabetes have shown that diet and exercise leading to weight loss consistently reduce the incidence of diabetes (Salas-Salvado et al., 2011).

**Exercise**

Physical activity is defined as the total of planned and repetitive movements of skeletal muscles, which are performed using energy. The beneficial effects of exercise in patients with diabetes have been well recognized. The beneficial role of physical exercise on glycemic control in patients with type 2 diabetes mellitus has been confirmed by several controlled trials including both aerobic and resistance exercise protocols. Exercise has been shown to increase insulin sensitivity, lower blood sugar levels, reduce body fat and improve physical fitness. (Konig and Berg, 2012)

**Pharmacological treatment**

*Oral Anti-Diabetic Medications*

- Anti-diabetic drugs (alpha glucosidase inhibitor, metformin, thiazolidine) to treat intravenous glucose tolerance (IGT) suppress the risk of developing type 2 diabetes (Chiasson et al., 2002).

- Sulphonyl urea drugs, metformin, and insulin are effective in controlling both microvascular disease and macrovascular disease, and earlier intervention is essential to the control of macrovascular disease (Cade, 2008).
• Comprehensive intervention including blood pressure and lipid management is extremely effective in controlling vascular complications and reducing mortality rate (Gupta and Guptha, 2010).

• Pioglitazone suppresses the recurrence of cardiovascular disorders.

![Figure 5: Oral Anti-Diabetic Medications and its mechanism](image)

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### Table 2: Experimental Animal Models for Diabetes Mellitus

<table>
<thead>
<tr>
<th>Diabetic animal models</th>
<th>Obese</th>
<th>Non-Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Spontaneous or genetically derived</td>
<td>• Ob/ob mouse</td>
<td>• Cohen diabetic rat</td>
</tr>
<tr>
<td></td>
<td>• db/db mouse</td>
<td>• GK rat</td>
</tr>
<tr>
<td></td>
<td>• KK mouse</td>
<td>• Torri rat Non obese</td>
</tr>
<tr>
<td></td>
<td>• KK/A^2 mouse</td>
<td>C57BL/6</td>
</tr>
<tr>
<td></td>
<td>• NZO mouse</td>
<td>• (Akita) mutant mouse</td>
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<tr>
<td></td>
<td>• NONcNZO10 mouse</td>
<td>• ALS/Lt mouse</td>
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<td></td>
<td>• TSOD mouse</td>
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<td></td>
<td>• M16 mouse</td>
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<tr>
<td></td>
<td>• Zucker fatty rat</td>
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<td></td>
<td>• ZDF rat</td>
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<tr>
<td></td>
<td>• SHR/N-cp rat</td>
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</tr>
<tr>
<td></td>
<td>• JCR/LA-cp rat</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• OLETF rat</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Obese Rhesus monkey</td>
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</tbody>
</table>
II. Diet/nutrition induced
- Sand rat
- C57BL/6J mouse
- Spiny mouse

III. Chemically induced
- GTG treated obese mice
- ALX or STZ adult rats, mice etc.
- Neonatal STZ rat

IV. Surgically induced
- VMH lesioned dietary obese diabetic rat
- Partial pancreatectomized animals e.g. Dog, primate, pig and rats

V. Transgenic/Knock-out
- $\beta$1 receptor knock-out mouse
- Uncoupling protein (UCP1) knock-out mouse
- Transgenic or knock-out mice involving diabetic animals genes of insulin and insulin receptor and its components of downstream insulin signaling e.g. IRS-1, IRS-2, GLUT-4, PTP-1B and others.
- PPAR-$\gamma$ tissue specific knockout mouse.
- Glucokinase or GLUT 2 gene knockout mice.
- Human islet amyloid polypeptide overexpressed rat (HIP rat)

<table>
<thead>
<tr>
<th>Model Category</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Spontaneous diabetic animals</td>
<td>Development of type 2 diabetes is of spontaneous origin involving genetic factors and the animals develop characteristic features resembling human type 2 diabetes</td>
<td>Highly inbred, homogenous and mostly monogenic inheritance and development of diabetes is highly genetically determined unlike heterogeneity seen in humans</td>
</tr>
<tr>
<td></td>
<td>Mostly of inbred animal models in which the genetic background is homogenous</td>
<td>Limited availability and expensive for the diabetes study</td>
</tr>
</tbody>
</table>

Table 3: Advantages and disadvantages of type 2 diabetic animal models

KK, Kuo Kondo; KK/$A'$, yellow KK obese; VMH, ventromedial hypothalamus; ZDF, Zucker diabetic fatty; NZO, New Zealand obese; TSOD, Tsumara Suzuki obese diabetes; SHR/N-cp, spontaneously hypertensive rat/NIH-corpulent; JCR, James C Russel; OLETF, Otuska Long Evans Tokushima fatty; GTG, gold thioglucose; ALX, alloxan; STZ, streptozotocin; GLUT - glucose transporter; IRS, insulin receptor substrate; GK, Goto-Kakizaki; PPAR, Peroxisome proliferator activated receptor, PTP, phosphotyrosine phosphatase; ALS, alloxan sensitive (Srinivasan and Ramarao, 2007).
and environmental factors can be controlled, allowing genetic dissection of this multifactorial disease easy.

Variability of results perhaps minimum and require small sample size.

Mortality due to ketosis problem is high in case of animals which brittle pancreas (db/db, ZDF rat *P. obesus*, etc.) and require insulin in latter stage for survival.

Require sophisticated maintenance.

| 2. Diet/ Nutrition induced diabetic animals | Develop diabetes associated with obesity as a result of over nutrition as in diabesity syndrome of human population.
Toxicity of chemicals on other body vital organs can be avoided. |
| 3. Chemical induced diabetic animals | Selective loss of pancreatic β-cells (Alloxan/ STZ) leaving other pancreatic alpha and delta cells intact.
Residual insulin secretion makes the animals live long without insulin treatment.
Ketosis and resulting mortality is relatively less.
Comparatively cheaper, easier to develop and maintain. |
| | Mostly require long period of dietary testament.
No frank hyperglycemia develops upon simple dietary treatment in genetically normal animals and hence become not suitable for screening anti diabetic agents on circulating glucose parameter. |
| | Hyperglycemia develops primarily by direct cytotoxic action on the β-cells and insulin deficiency rather than consequence of insulin resistance.
Diabetes induced by chemicals is mostly is less stable and at times reversible because of the spontaneous regeneration of β-cells. Hence, care must be taken to assess the pancreatic β-cell function during long-term experiments.
Chemical produce toxic actions on other body organs as well besides its cytotoxic action on β-cells. |
<table>
<thead>
<tr>
<th>4. Surgical diabetic animals</th>
<th>Avoids cytotoxic effects of chemical diabetogens on other body organs</th>
<th>Involvement of cumbersome technical and post operative procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resembles human type 2 diabetes due to reduced islet β-cell mass</td>
<td>Occurrence of some other digestive problems (as a result of part of excision of exocrine portion (deficiency of amylase enzyme))</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dissection of alpha islets (glucagon secreting cells) two along with β-cells leading to problems in counter regulatory response to hyperglycemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mortality is comparatively higher</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>5. Transgenic/ Knockout diabetic animals</th>
<th>Effect of single gene or mutation on diabetes can be investigated in vivo</th>
<th>Highly sophisticated and costly procedure for the production and maintenance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dissection of complex genetics of type 2 diabetes become easier</td>
<td>Expensive for regular screening experiments</td>
</tr>
</tbody>
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**EXPERIMENTAL INDUCTION METHODS FOR DIABETES MELLITUS**

The existence of experimental animal model of disease aids not only in the understanding the pathophysiology of such a disease, but also in the development of drugs for its treatment. Due to its high prevalence and potential deleterious effects on a patient’s physical and psychological state,
diabetes is a major medical concern (Macedo et al., 2002). The disease remains incurable and can only be controlled with drugs.

Over the years, several animal models have been developed for studying various aspects in the initiation and development of diabetes mellitus or testing the efficacy of anti-diabetic agents. These models include chemical, surgical (pancreatectomy) and genetic manipulations in several animal species to induce experimental diabetes mellitus. Experimental animal models provide an opportunity to study the genetic and environmental factors that may influence the development of the disease and establishment of its complications, and thus gain new information about its handling and treatment in humans.

Animal models have enormously contributed to the study of diabetes mellitus. The various experimentally induced rodent models for type 2 diabetes can be conveniently classified as follows;

**Surgical model of diabetes mellitus**

Surgical model is used to induce diabetes and it involves either partial or total removal of the pancreas (pancreatectomy). Few researchers have employed this model to explore effects of natural products with animal species such as rats, pigs, dogs and primates (Choi et al., 2004, Rees and Alcolado, 2005; Masiello, 2006). Limitation to this technique include high level of
technical expertise and adequate surgical room environment, major surgery and high risk of animal infection, adequate post-operative analgesia and antibiotic administration, supplementation with pancreatic enzymes to prevent malabsorption and loss of pancreatic counterregulatory response to hypoglycemia. More recently, partial pancreatectomy has been employed, but larger resection (more than 80% in rats) is required to obtain mild to moderate hyperglycemia. In this case, small additional resection can result in significant hypoinsulinemia (Choi et al., 2004). Masiello, (2006) investigated the action of relative glucose uptake in various tissues of 90% pancreatectomized rats by using either hyperglycemic or euglycemic hyperinsulinemic clamp methodologies. This experimental design permits to evaluate if the compound has some effect upon both resistance to and secretion of insulin.

**GENETIC MODEL OF DIABETES**

*Spontaneously develop diabetic rats*

These models permit the evaluation of the effect of natural products in animal models without the interference of the side effects induced by chemical drugs like Alloxan and Streptozotocin. The spontaneously diabetic Goto-Kakizaki rat model, which is a genetic lean model of type 1 diabetes originating from selective breeding over many generations of glucose-intolerant non-diabetic Wistar rats (Chen and Wang, 2005). Regarding type 1 diabetes models, the mouse typically presents hyperglycemia between 12 and 30 weeks of age, whereas in BB rats, it occurs around 12 weeks of age. One
great advantage of these models is that they can be employed as model of atherosclerosis which represents the long term complication of diabetes mellitus and tested against several natural products (Wu and Huan, 2007). Mutant strains of obese diabetic mice are available such as C57BL/KsJ-db/db. With this model, it is possible to test for effects of plant extracts on blood sugar, body weight, insulin production and insulin resistant.

**Chemical induction of diabetes mellitus**

The majority of studies published in the field of ethnopharmacology between 1996 and 2006 employed this model. Both alloxan and streptozotocin exert their diabetogenic action when they are administered parenterally (intravenously, intraperitoneally or subcutaneously). The dose of these agents required for inducing diabetes depends on the animal species, route of administration and nutritional status. However, streptozotocin is by far the most frequently used drugs and this model has been useful for the study of multiple aspects of diabetes mellitus. (Federiuk et al., 2004).

**Alloxan model of diabetes mellitus**

Alloxan, a well-known diabetogenic agent is widely used to induce type 2 diabetes in animals (Viana et al., 2004). Alloxan (2,4,5,6-tetraoxypyrimidine; 5,6-dioxyuracil) was originally isolated in 1818 by Brugnatelli. Wohler and Liebig used the name ‘Alloxan’ in 1838 and described its synthesis by Uric acid oxidation. Dunn et al (1943) studied the effect of alloxan and reported a specific necrosis of pancreatic islets.
Mechanism of action

The action of alloxan in the pancreas is preceded by its rapid uptake by the beta cells. Rapid uptake by insulin secreting cells has been proposed to be one of the important features determining alloxandiabetogenecity (Kliberet al., 1996). Another aspect concerns the formation of reactive oxygen species. A similar uptake of alloxan also takes place in liver. However, the liver and other tissues are more resistant to reactive oxygen species in comparison to pancreatic beta cells and this resistance protects them against the alloxan toxicity. The formation of reactive oxygen species is preceded by the alloxan reduction. In the beta cells of pancreas, its reduction occurs in the presence of different reducing agents. The result of alloxan reduction is the formation of Dialuric acid.

The reaction between alloxan and dialuric acid is a process in which intermediate alloxan radicals are formed. One of the targets of the reactive oxygen species is DNA of pancreatic islets. Its fragmentation takes place in beta cells exposed to alloxan. DNA damage stimulates poly ADP-ribosylation, a process participating in DNA repair. Therefore chemicals
rendering anti-oxidative properties and inhibiting poly ADP-ribosylation can attenuate alloxan toxicity (Takasu et al., 1991).

The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of betacells (Szkudelki, 2001). Thus alloxan induced diabetes mellitus serves as a pathological biomodel for testing a substance with supposed antioxidant activities in vivo (Bartosikova et al., 2003). The increase in oxygen free radicals in diabetic conditions is mainly because of the effect of the diabetogenic agent alloxan. However, the requirement of large amounts of alloxan and its reversible nature restricts its usage in the induction of diabetes in experimental animals.

**Streptozotocin model of diabetes mellitus**

Streptozotocin(2-deoxy-2-([methyl(nitroso)amino]carbonyl)amino)-β-D-glucopyranose is a chemical that is specifically toxic to the insulin-producing beta cells of the pancreas in mammals. The drug was discovered in the strain of the soil microbe, *Streptomyces achromogenes*. Induction of experimental diabetes in rats using streptozotocin (STZ) is very convenient and simple to use. STZ injection leads to the degeneration of the beta cells - islets of Langerhans (Rakietien et al., 1963). Clinically, symptoms of diabetes are clearly seen in rats within 2-4 days following single intravenous or intraperitoneal injection of 60mg/kg STZ.
**Streptozotocin**

**Mechanism of action:**

STZ is taken up by the pancreatic beta cells via glucose transporter GLUT2. Intracellular action of STZ results in changes of DNA in pancreatic beta cells comprising its fragmentation (Szkudelski, 2001). Recent experiments have proved that the main reason for the STZ induced beta-cell death is alkylation of DNA. The alkalyting activity of STZ is related to its nitrosourea moiety, especially at the -O6 position of guanine. After STZ injection to rats, different methylated purines were found in tissues of those animals (Bennett and Pegg, 1981).

On the other hand, STZ is a nitric oxide (NO) donor and NO was found to bring about the destruction of pancreatic islet cells; it was proposed that these molecules contribute to STZ induced DNA damage. The participation of NO in the cytotoxic effect of STZ was confirmed in several experiments (González et al., 2000). STZ is however not a spontaneous nitric
oxide donor. This molecule is liberated when STZ is metabolized inside cells, but NO synthase is not required for the diabetogenic effect. However, the results of several experiments provide the evidence that NO is not the only molecule responsible for the cytotoxic effect of STZ (Szkudelski, 2001).

STZ was found to generate excessive amounts of reactive oxygen species, which also contributes to DNA fragmentation and evokes other deleterious changes in the cells. The formation of superoxide anions results from both STZ action on mitochondria and increased activity of xanthine oxidase. It was demonstrated that STZ inhibits the Kreb’s cycle and substantially decreases oxygen consumption by mitochondria. These effects strongly limit mitochondrial ATP production and causes depletion of the nucleotide in beta cells. Restriction of mitochondrial ATP generation is partially mediated by NO (Kolluru et al., 2012).

It can be stated that potent alkylating properties of STZ are the main reason of its toxicity. However, the synergistic action of both NO and ROS may also contribute to DNA fragmentation and other changes caused by STZ. NO and reactive oxygen species can act separately or form the highly toxic peroxynitrate. Therefore, intracellular antioxidants or NO scavengers substantially attenuate STZ toxicity.

**High-fat diet fed models**

A number of researchers used the approach of high fat feeding to develop rodent models of T2D using different strains of mice or rats and
different amounts of dietary calories from fat. Obesity is one of the major factors for the development of T2D, and obesity usually develops when rodents are fed with diet containing high amounts of fat (40–60% of the total calories). When this model was developed using C57BL/6 J strain insulin resistance but no beta cell failure was observed, and that insulin resistance in the C57BL/6 J strain was compensated by a marked beta cell proliferation (Reuter et al., 2007).

The high-fat diet models are usually characterized by overweight, obesity, impaired glucose tolerance and insulin resistance. Initiation of fat feeding at 6-8 weeks produced effective obesity in this model. The C57BL/6 J mice are typically fed with diet containing 40–60% of calories from fat, approximately eight times higher fat content than that of control mice for 8–16 weeks. The longer duration of fat-feeding enhances the clinical features of insulin resistance, impaired glucose tolerance, revealing elevated serum insulin and glucose, abnormal lipid profile, and mild to moderate hyperglycemia. The approach of HF diet feeding to induce T2D has also been implicated to out bred Sprague–Dawley (SD) rats as it has remarkable sensitivity to HF diet to induce insulin resistance and diabetes as opposed to some other strains of rats (Paoli et al., 2013). At a longer stage of feeding, this model can also develop hyperlipidemia and hyperinsulinemia leading to T2D; however, the major disadvantage of this model is also the duration of time (>10 weeks) required to induce the all major pathogenesis of T2D particularly
hyperglycemia and insulin resistance, which is not suitable for many researchers, as this increases the cost of the experiment.

**Fat fed streptozocin model**

Many studies have reported that the rats fed with highfat diet (HFD) develop insulin resistance but not frank hyperglycemia or diabetes. It is suggested that the HFD might be a better way to initiate the insulin resistance which is one of the important clinical features of type 2 diabetes. At the same time, STZ is widely used to reproducibly induce both insulin-dependent and non-insulin-dependent diabetes mellitus presently by inducing β-cell death through alkylation of DNA (Chen and Wang, 2005). Although high-dose STZ severely impairs insulin secretion mimicking type 1 diabetes, low-dose STZ has been known to induce a mild impairment of insulin secretion which is similar to the clinical feature of the later stage of type 2 diabetes (Zhang et al., 2008). Therefore, investigators have started to develop a rat model by feeding the animal with high-fat diet following low-dose STZ that would closely mimic the natural history of the disease events (from insulin resistance to β cell dysfunction) as well as metabolic characteristics of human type 2 diabetes.

Recently, a similar, but modified approach of the model was attained with the administration of a HF diet containing 58% calories from lard to Sprague Dawley rats for 2 weeks prior to STZ injection (i.p.) with a varying dosage of 25, 35, 45, 55 mg/kg BW and continued *ad-libitum* feeding
of HF diet throughout the experimental period. The animals injected with 35 mg/kg STZ dose showed frank hyperglycemia, significantly elevated total serum cholesterol, and serum triglycerides. Serum insulin concentration was significantly lower in this group compared to the rats fed only a HF diet (Zhang et al., 2005; Srinivasan et al., 2005; Zhang et al., 2008). Advantageously, this model was also sensitive to two anti-diabetic drugs confirming its suitability as a model for T2D. Hence, HFD- low dose STZ induced experimental model appears to be a good choice for use as a rodent model for T2D either for rapid and routine pharmacological screening of antidiabetic drugs and natural products.

**METAL COMPLEXES IN BIOLOGY**

Metals have played an important role in medicine for years, ever since humans have walked the planet. The use of metals in therapeutic drugs becomes increasingly important over the last couple of decades result in a variety of exciting and valuable metallopharmaceutical drugs such as cis-platin and aururanofin as anticancer and antiarthritic drugs, respectively (Jamieson and Lippard, 1999). Many metals are essential in our diets in varying quantities, although people have recently realized their significance. This could probably be attributed to our increased awareness of personal and families’ health and increased media involvement in our life. Metals perform a wide variety of tasks such as carrying oxygen throughout the body and shuttling electrons. The intentional introduction of a metal ion into a biological system will be for either therapeutic or diagnostic purpose. As was noted by Peter Sadler some
years ago, most of the elements of periodic table up to and including bismuth, with an atomic number of 83, have potential uses in the design of new drugs and diagnostic agents. Sadler also pointed out that medicinal inorganic chemistry provides active metal complexes, active metal ions, or even active ligands, as potential agents (Sadler, 1991).

Many biologically active compounds used as drugs possess modified pharmacological and toxicological potentials when administered in the form of metal-based compounds (Louie and Meade, 1999; Ming, 2003; Timerbaev et al., 2006). Many metallic elements play a crucial role in living systems. A characteristic of metals is that they easily lose electrons from the familiar elemental or metallic state to form positively charged ions, which tend to be soluble in biological fluids. It is in this cationic form that metals play their role in biology. Whereas metal ions are electron deficient, most biological molecules such as proteins and DNA are electron rich. The attraction of these opposing charges leads to a general tendency for metal ions to bind to and interact with biological molecules. This same principle applies to the affinity of metal ions for many small molecules and ions crucial to life, such as \( \text{O}_2 \). Given this wide scope for the interaction of metals in biology, it is not surprising that natural evolution has incorporated many metals into essential biological functions. Metals perform a wide variety of tasks such as carrying oxygen throughout the body and shuttling electrons. Hemoglobin, an iron-containing protein that binds to oxygen through its iron atom, ferries this vital molecule to body tissues. Metal ions such as zinc provide the structural
framework for the zinc fingers that regulate the function of genes in the nuclei of cells. Similarly, calcium-containing minerals are the basis of bones, the structural framework of the human body (Holm et al., 1996).

Zinc is a natural component of insulin, a substance crucial to the regulation of sugar metabolism. Metals such as copper, zinc, iron, and manganese are incorporated into catalytic proteins the metalloenzymes which facilitate a multitude of chemical reactions needed for life (Solomon et al., 1996). The increasing knowledge of the biological activities of simple metal complexes guided many researchers to the development of promising chemotherapeutic compounds which target specific pathological processes. These studies involve both synthetic and natural products, in association with essential metal ions. In the development of insulin-mimetic or insulin-sensitizing agent, we have synthesized a low molecular weight metal complex by using naturally occurring ligand to enhance the lipophilicity, membrane transport and bioavailability (Sendrayaperumal et al., 2014).

ZINC

Zinc with atomic number 30, atomic weight 65.39 and oxidation state II is an essential element in all living systems and plays a structural role in many proteins and enzymes. It is recognized that transcription factors regulate gene expression and the essential feature is binding to a regulatory protein in the recognition sequence of the gene. Many proteins have
been found to have a zinc-containing motif that serves to bind DNA embedded in their structure. In the relevance of zinc to DM, zinc is known to be present in insulin, coordinated by three nitrogen atoms from histidine and three water molecules in an irregular octahedral environment, which is also believed to have a functional structure (Sakurai et al., 2002). Surprisingly, zinc was found to have important physiological and pharmaceutical functions involving insulin-mimetic activity. In 1980, Coulston and Dandona first reported the insulin-mimetic activity of zinc ion, in which administration of ZnCl₂ to STZ-rats or ob/ob mice normalized their high blood glucose levels. However, they used high doses or long-term (8 weeks) in zinc(II) ion administration. Following this observation, several research groups attempted to confirm the insulin-mimetic activity of the zinc ion (Coulston and Dandona, 1980; May and Contoreggi, 1982; Shisheva et al., 1992).

**Insulin, diabetes and zinc**

A relationship between Zn, pancreatic function and diabetes was suggested almost 70 years ago. Crystalline insulin was isolated in 1926, using a highly buffered solution containing several substances as the crystallising medium (Abel, 1926). The very nature of the substance promoting crystallization and the mechanisms of crystal formation remained unclear despite it being known that the pancreas contains high amounts of Zn. A few years later, Scott discovered that adding Zn to a phosphate-buffered solution containing insulin induced the formation of characteristic rhombohedral insulin crystals. Because of the close association between insulin and Zn,
Scott’s next idea was to estimate the Zn content in the pancreas of a series of normal and diabetic individuals (Scott and Fisher, 1938). Interestingly, he found that the amount of Zn contained in the pancreas of diabetics is only one-half that of healthy subjects, while there was no difference in the liver Zn concentration, raising the possibility that at least part of the Zn in the pancreas could be concerned with the storage of insulin. This forms the major understanding of the link between insulin and Zn.

A second breakthrough in the diabetes field came from the discovery of the structure of insulin. Following Scott’s discovery that the insulin preparations from different species had the same effect, it has been suggested that different insulin’s behave as a single molecule in solubility studies, despite some differences in some amino acids (Sanger, 1949). In 1955, Sanger and colleagues determined that conserved amino acids and important disulfide bridges might be implicated in insulin activity, since insulin was inactivated by any treatment affecting those sulfur bonds (Brown et al., 1955). After many years of research that brought new insights to our understanding of the structure of insulin, including the determination of single-chain amino acid composition and X-ray photographs of single insulin crystals, (Adamset al., 1969) eventually determined the crystal structure at a resolution of 2.8 Å Angstrom. They showed that the crystal was formed by six insulin molecules and two Zn atoms and determined the intramolecular Zn coordination spheres (Coulston and Dandona, 1980).

**Zinc transporters**
It is now well established that cells control the uptake and excretion of Zn\textsuperscript{2+} through two different families of proteins: the SLC39A and SLC30A genes, which encode for ZIP and ZnT, respectively (Lichten and Cousins, 2009). To date, up to twenty-four Zn transporters have been described, most of which have relevance to clinical science. Some transporters have ubiquitous expression, while others are restricted to a few tissues, which led to the question of why there are so many Zn transporters, compared with those needed for other ions such as Cu or Fe (Kambe et al., 2014). Such a large panel of ZnT and ZIP transporters both serves a housekeeping role in cellular Zn homeostasis and participates in cell signaling (Lemaire et al., 2012).

Zn transporters play important physiological roles, for example, during embryogenesis, cell division and migration, and have a specific role in different organ systems, including but not restricted to the brain, immune system, skin and pancreas (Yan et al., 2012). The Zn transporter ZIP4, expressed at the apical surface of intestinal enterocytes and visceral endoderm cells, responds to Zn levels and translocates from cytoplasmic vesicles to the plasma membrane to enhance Zn uptake during Zn deficiency, suggesting that Zn regulated intracellular trafficking of Zn transporters is an important mechanism for the control of dietary Zn absorption, and cellular Zn homeostasis (Kim et al., 2004). Other members of the ZIP family have been shown to be activated posttranslationally by phosphorylation, and are strongly implicated in cell signaling (Taylor et al., 2012).
In response to extracellular Zn orepidermal-growth-factor/ionomycine treatment, the endoplasmic reticulum Zn transporter ZIP7 is phosphorylated on conserved residues by protein kinase CK2, leading to the release of intracellular Zn stores and subsequent activation of protein kinase B (Akt), and extracellular signal-regulated kinases 1 and 2 (ERK1/2) (Taylor et al., 2012). ZIP7 is therefore a key protein for Zn signaling during proliferative responses and cell migration. A member of the ZnT family, ZnT1, levels of which rapidly increase after global ischaemic injury, is associated with long-life (L)-type Ca channels, thus leading to downstream activation of ERK and heart protection after ischaemia–reperfusion injury (Beharier et al., 2012). Indeed, at the level of the organism, Zn transporters play a crucial role in maintaining adequate Zn homeostasis in all organs (Bosco et al., 2010).

**Zinc Finger Proteins**

Zinc finger (ZnF) proteins are a massive, diverse family of proteins that serve a wide variety of biological functions. Due to their diversity, it is difficult to come up with a simple definition of what unites all ZnF proteins; however, the most common approach is to define them as all small, functional domains that require coordination by at least one zinc ion (Laity et al., 2001). The zinc ion serves to stabilize the integration of the protein itself, and is generally not involved in binding targets. The “finger” refers to the secondary structures (α-helix and β-sheet) that are held together by the Zn ion. Zinc finger containing domains typically serve as interactors, binding DNA, RNA, proteins or small molecules (Laity et al., 2001).
**ZnF Protein Families**

Cys2His2 was the first domain discovered (also known as Krüppel-type). It was initially discovered as a repeating domain in the IIIA transcription factor in Xenopus laevis (Brown et al., 1985). IIIA has nine repeats of the 30 amino acids that make up the Cys2His2 domain. Each domain forms a left-handed $\beta\beta\alpha$ secondary structure, and coordinates a Zn ion between two cysteines on the $\beta$-sheet hairpin and two histidines in the $\alpha$-helix, hence the name Cys2His2 (Lee et al., 1989). These resides are highly conserved, as well as a general hydrophobic core that allows the helix to form. The other residues can show great sequence diversity. Cys2His2 zinc fingers that bind DNA tend to have 2-4 tandem domains as part of a larger protein. The residues of the alpha helices form specific contacts with a specific DNA sequence motif by “reading” the nucleotides in major groove of DNA (Pavletich and Pabo, 1991; Elrod-Erickson et al., 1996). Cys2His2 proteins are the biggest group of transcription factors in most species. Non-DNA binding proteins can have much more flexible tertiary structure. Examples of Cys2His2 proteins include the Inhibitor of Apoptosis (IAP) family of proteins and the CTFC transcription factor (Miller et al., 1985).

Treble clef fingers are a very diverse group of ZnF proteins both in terms of structure and function. What makes them a family is a shared fold at their core that looks a little like a musical treble clef, especially if you squint (Grishin, 2001). Most treble clef finger motifs have a $\beta$ hairpin, a variable loop region, a $\beta$ hairpin, and an $\alpha$ helix. The “knuckle” of the $\beta$ hairpin and
the α helix contain the Cys-x-x-Cys sequence necessary to coordinate the Zn ion. Treble clef fingers often form the core of protein structures, for example the L24E and S14 ribosomal proteins and the RING finger family.

Zinc ribbons are a little less structurally complex than the other two major groups. Zinc ribbons contain two zinc knuckles, often β hairpins, coordinating a zinc ion via a two Cys residues separated by 2-4 other residues on one knuckle, and a Cys-x-x-Cys on the other (Hahn and Roberts, 2000). Examples of zinc ribbon-containing proteins include the basal transcription factors TFIIS and TFIIB that for a complex with RNAPII to bind DNA, and the Npl4 nuclear core protein that uses zinc ribbon to bind ubiquitin (Alam et al., 2004). Cys2His2, treble clef fingers, and zinc ribbons form the majority of zinc fingers, but there are several other smaller groups that don’t fit neatly into these three.

**Metallothionein, zinc and oxidative stress**

Metallothioneins (MTs) belong to the group of intracellular cysteine-rich, metal-binding proteins that have been found in bacteria, plants, invertebrates and vertebrates (Coyle et al., 2002; Vasak, 2005). These low molecular weight cysteine-rich proteins have been continuously studied in all aspects, including physical, chemical and biochemical properties. These unique proteins are involved in diverse intracellular functions (Davis and Cousins, 2000), but their role in the detoxification of heavy metals and in
themaintenance of essential metal ion homeostasis, which is due to their high affinity for these metals, is mostly investigated (Templeton and Cherian, 1991). Based on structural models, it can be assumed that the MT molecule is composed of two binding domains, \( \alpha \) and \( \beta \), which are composed of cysteine clusters. The N-terminal part of the peptide is designated as \( \beta \)-domain and has three binding sites for divalent ions, and the C-terminal part (the \( \alpha \)-domain) has the ability to bind four divalent metal ions.

The binding of zinc to MTs has proven to be physiologically relevant. Several studies have produced strong evidence to support the idea that MTs function as zinc chaperones for the regulation of gene expression and activity of proteins, such as metalloproteins and metal-dependent transcription factors. Zinc(II) itself causes an increase in the major zinc-binding protein metallothionein. The induction of MT expression is induced through metal regulatory transcription factor 1 (MTF-1), a transcription factor, which directly responds to increased levels of free zinc(II) (Wang et al., 2010). Thus, MTF-1 binds the metal-responsive element of the MT gene and initiates MT transcription (Andrews, 2000). This autoregulatory loop maintains narrow optimal limits of intracellular zinc(II) and helps to reduce generated oxidative stress.

MEDICINAL PLANTS

For centuries, people have used plants for healing. Plant products – as parts of foods or botanical potions and powders – have been used with
varying success to cure and prevent diseases throughout history. Written records about medicinal plants date back at least 5000 years to the Sumerians and archeological records suggest even earlier use of medicinal plants (Raskin et al., 2002). The strong historic bond between plants and human health began to unwind in 1897, when Friedrich Bayer and Co. introduced synthetic acetyl salicylic acid (aspirin) to the world. Aspirin is a safer synthetic analogue of salicylic acid, an active ingredient of willow bark, and was discovered independently by residents of both the new and old worlds as a remedy for aches and fevers (Pierpoint, 1994).

Rediscovery of the connection between plants and health is responsible for launching a new generation of botanical therapeutics that include plant-derived pharmaceuticals, multicomponent botanical drugs, dietary supplements, functional foods and plant-produced recombinant proteins. Many of these products will soon complement conventional pharmaceuticals in the treatment, prevention and diagnosis of diseases, while at the same time adding value to agriculture. Such complementation can be accelerated by developing better tools for the efficient exploration of diverse and mutually interacting arrays of phytochemicals and for the manipulation of the plant’s ability to synthesize natural products and complex proteins.

Phytochemicals such as flavonoids, terpenoids, saponins, alkaloids and anthraquinones are plant-derived secondary metabolites found rich in fruits and vegetables (Havsteen, 2002). They are important constituents of the non-energetic part of the human diet and are thought to promote optimal
health, partly via their antioxidant effects in protecting cellular components against reactive oxygen species. Flavanoids are classified as flavone, flavonol, flavanone, isoflavone, anthocyanidin and proanthocyanidins. Among these classes, flavonol is known to chelate metal ions with the presence of multiple hydroxyl groups and α-hydroxycarbonyl group.

Flavonoids are known to exhibit a multitude of biological activities such as antioxidant, antibacterial, antiinflammatory, antiallergic, vasodilatory, anticarcinogenic (Havsteen, 2002). Due to their abundance in dietary products and their potential pharmacological and nutritional effects, the flavonoids are of considerable interest for drug discovery as well as health food supplement. Several flavonoids such as Kaemferol, Rutin and Quercitin are known to possess antidiabetic properties (Youl et al., 2010; Zhang and Liu 2011). Recently, we have reported the antidiabetic and antioxidant potentials of flavonoids such as fisetin, gossypin, rosmarinic acid in streptozotocin induced experimental diabetes in rats (SriramPrasath and Subramanian 2011; Venkatesan and Sorimuthupillai, 2012; Jayanthy and Subramanian 2014).

**ZINC-MORIN COMPLEX**

Most of the metal complexes so far investigated for their possible anti-diabetic activity were poorly absorbed in their inorganic forms and required high doses which have been associated with undesirable side effects. An important advance in the use of zinc salts as insulin mimics has been the
development of various ligands in order to reduce the toxicity of zinc and also to improve the stability, absorption, utilization and efficiency.

Among these classes, flavonol is known to chelate metal ions with the presence of multiple hydroxyl groups and α-hydroxycarbonyl groups (Rice-Evans and Miller, 1996). Morin, a natural bioflavonol, was originally isolated from the plants of the *Moraceae* family and it is abundantly present in *Psidium guajava* L. It is reported to have several pharmacological properties including antioxidant, anti-inflammatory, nephroprotective, chemoprotective as well as insulin mimetic activity (Arima and Danno, 2002; Paoli et al., 2013). For the development of a novel zinc complex, in the present study, we have synthesized a zinc complex using morin and evaluated its antidiabetic efficacy in HFD-STZ induced type 2 diabetes in experimental rats.


non-alcoholic fatty liver disease (NAFLD). *LipidsHealthDis* **9**: 42.


