INTRODUCTION
1. INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (World Health Organization [WHO], 2006). According to the WHO projections, the prevalence of diabetes is likely to increase by 35% by the year 2025 (Boyle et al., 2001).

Several pathogenic processes are involved 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. Deficient 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. An 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 (American Diabetes Association, 2006).

1.1. Etiologic classification of diabetes mellitus

1.1.1. Type 1 diabetes mellitus

Type 1 diabetes is characterized by an absolute insulin insufficiency caused by the immunological destruction of pancreatic β-cells, which
produce and secrete insulin, and it accounts for $\geq 10\%$ of all cases of diabetes.

1.1.2. Type 2 diabetes mellitus

Type 2 diabetes is more complex in etiology and is characterized by a relative insulin deficiency, reduced insulin action, and insulin resistance of glucose transport in skeletal muscle and adipose tissue.

1.1.3. Other specific types:

i) Genetic defects of $\beta$-cell function

ii) Genetic defects in insulin action

iii) Diseases of the exocrine pancreas

iv) Endocrinopathies

v) Drug- or chemical-induced diabetes

vi) Infections

vii) Uncommon forms of immune-mediated diabetes

viii) Other genetic syndromes sometimes associated with diabetes

(American Diabetes Association, 2006)

1.1.4. Gestational diabetes mellitus

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. The definition applies regardless of whether insulin or only diet modification is used for treatment or whether the condition persists after pregnancy. It does not exclude the possibility that unrecognized glucose intolerance may have antedated or begun concomitantly with the pregnancy. GDM represents nearly 90$\%$ of all pregnancies complicated by diabetes. Deterioration of
glucose tolerance occurs normally during pregnancy, particularly in the 3rd trimester (American Diabetes Association, 2006).

1.2. Criteria for the diagnosis of diabetes mellitus

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

2. Fasting plasma glucose (FPG) ≥126 mg/dl (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 hrs.

3. 2 hrs postload glucose ≥200 mg/dl (11.1 mmol/L) during an oral glucose tolerance test (OGTT). The test should be performed as described by World Health Organization (WHO), using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water (American Diabetes Association, 2006).

1.3. Pathogenesis of diabetes mellitus

1.3.1. Type 1 diabetes mellitus

The pathophysiology of type 1 diabetes is thought to be due to an absolute insulin deficiency without significant insulin resistance that results in hyperglycemia, spontaneous ketosis and if untreated, diabetic ketoacidosis. This absolute insulin deficiency is thought to be caused by autoimmune β-cell destruction, triggered by unknown mechanisms. The hallmark of type 1 diabetes is the selective destruction of insulin-producing cells in the pancreas or insulitis. Type 1 diabetes results from a severe or absolute lack of insulin caused by a reduction in β-cell mass. The pathogenesis of IDDM is
slow, progressive immunological destruction of the β-cell mass by antigen specific cytotoxic T-lymphocytes augmented by cytokine release from macrophage and natural killer cells (Atkinson and Eisenbarth, 2001). It is also characterized by the presence of anti-GAD, islet cells or insulin antibodies (islet cell cytoplasmic antibodies, islet cell surface antibodies and specific antigenic targets of islet cells), which identify the autoimmune process that lead to β-cell destruction (Devendra et al., 2004)

1.3.2. Type 2 diabetes mellitus

Type 2 diabetes is characteristically a disease of the middle aged or elderly and usually begins insidiously. More than 90% of all identified cases of diabetes are classified as type 2 (Nathan et al., 1997). The pathogenesis of type 2 diabetes mellitus is multifactorial and complex, and it is influenced by both genetic and environmental factors. This metabolic disorder results from insulin resistance in target tissues and an impairment of pancreatic insulin secretion (Brandy et al., 2004). Analysis is complicated by the fact that hyperglycemia per se impairs both insulin secretion and insulin action.

1.4. Complications of diabetes mellitus

Diabetes gives rise to the development of numerous complications due to hyperglycemia. The likelihood of developing complications, whether acute or chronic, is ultimately a reflection of the level of blood sugar control. A large body of evidence indicates that good blood sugar control significantly reduces the development of complications. Therefore, monitoring and controlling the degree of hyperglycemia is critical to the prevention of the major diabetic complications.
1.4.1. Acute complications

Diabetics are susceptible to three major acute complications: hypoglycemia, diabetic ketoacidosis (primarily affects type 1 diabetics), and nonketogenic hyperosmolar syndrome (primarily affects type 2 diabetics).

1.4.1.1. Hypoglycemia

The problem of hypoglycemia is much more common in type 1 than type 2, because the type 1 diabetic is injecting insulin. Taking too much of insulin, missing a meal, or over-exercising can result in hypoglycemia. Daytime hypoglycemic episodes are usually recognized by the following symptoms: sweating, nervousness, tremor, and hunger. Night-time hypoglycemia may be without symptoms or manifest as night sweats, unpleasant dreams, or early morning headache (Pizzorno and Murray, 1995).

1.4.1.2. Diabetic ketoacidosis

Another acute complication more likely to occur in the type 1 diabetic is ketoacidosis, a condition caused by a lack of insulin leading to a buildup of ketoacids. If progressive, ketoacidosis can result in numerous metabolic problems and even coma. Since ketoacidosis is potentially a medical emergency, prompt recognition is imperative. The coma is usually preceded by a day or more of increased urination and thirst as well as marked fatigue, and nausea and vomiting (Pizzorno and Murray, 1995).

1.4.1.3. Non-ketogenic hyperosmolar syndrome

With a mortality rate of over 50%, non-ketogenic hyperosmolar syndrome constitutes a true medical emergency. It is usually the result of profound dehydration, secondary to deficient fluid intake or precipitating
events such as pneumonia, burns, stroke, a recent operation, or certain drugs such as phenytoin, diazoxide, glucocorticoids and diuretics. The onset of the syndrome may be insidious over a period of days or weeks, with symptoms of weakness, increased urination and thirst, and progressively worse signs of dehydration (weight loss, loss of skin elasticity, dry mucous membranes, rapid heart beat, and low blood pressure) (Pizzorno and Murray, 1995).

1.4.2. Chronic complications

The morbidity and mortality of diabetes is due to the development of both microvascular and macrovascular complications (Brownlee, 2001). Long-term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations, and charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction. Macrovascular complications including myocardial infarction, stroke and large vessel peripheral vascular disease are 2 to 4 times more prevalent in individuals with diabetes (Feldman, 2003).

1.4.2.1. Diabetic nephropathy

Diabetic nephropathy is the leading cause of kidney disease in patients starting renal replacement therapy and is associated with increased cardiovascular mortality (Valmadrid et al., 2000). Diabetic nephropathy affects \( \approx 40\% \) of type 1 and type 2 diabetic patients. It increases the risk of death, mainly from cardiovascular causes, and is defined by increased urinary albumin excretion (UAE) in the absence of other renal diseases.
Diabetic nephropathy has been didactically categorized into stages based on the values of UAE: microalbuminuria and macroalbuminuria. It is more prevalent among African Americans, Asians, and Native Americans than Caucasians (Young et al., 2003).

1.4.2.1.1. Risk factors

Diabetic nephropathy develops in, at most, 40% of patients with diabetes, even when high glucose levels are maintained for long periods of time. Furthermore, epidemiological and familial studies have demonstrated that genetic susceptibility contributes to the development of diabetic nephropathy in patients with both type 1 and type 2 diabetes (Gross et al., 2005).

1.4.2.1.2. Pathophysiology

Diabetes causes unique changes in kidney structure. Classic glomerulosclerosis is characterized by increased glomerular basement membrane width, diffuse mesangial sclerosis, hyalinosis, microaneurysm, and hyaline arteriosclerosis (Mauer et al., 1981). Tubular (Brito et al., 1998) and interstitial (Katz et al., 2002) changes are also present. Areas of extreme mesangial expansion called Kimmelstiel-Wilson nodules or nodular mesangial expansion are observed in 40-50% of patients developing proteinuria. Micro- and macroalbuminuric patients with type 2 diabetes have more structural heterogeneity than patients with type 1 diabetes (Fioretto et al., 1996).
1.4.2.1.3. Prevention or treatment

The basis for the prevention of diabetic nephropathy is the treatment of its known risk factors: hypertension, hyperglycemia, smoking, and dyslipidemia (Gross et al., 2005).

1.4.2.2. Diabetic retinopathy

Diabetic retinopathy is the most severe of the several ocular complications of diabetes (Frank, 2004). It progresses from mild nonproliferative abnormalities, characterized by increased vascular permeability, to moderate and severe nonproliferative diabetic retinopathy (NPDR), characterized by vascular closure, to proliferative diabetic retinopathy (PDR), characterized by the growth of new blood vessels on the retina and posterior surface of the vitreous. Macular edema, characterized by retinal thickening from leaky blood vessels, can develop at all stages of retinopathy. Pregnancy, puberty, blood glucose control, hypertension, and cataract surgery can accelerate these changes (Fong et al., 2004).

1.4.2.2.1. Risk factors

The duration of diabetes is probably the strongest predictor for the development and progression of retinopathy. Among younger-onset patients with diabetes in the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR), the prevalence of any retinopathy was 8% at 3 years, 25% at 5 years, 60% at 10 years, and 80% at 15 years. The prevalence of PDR was 0% at 3 years and increased to 25% at 15 years. The incidence of retinopathy also increased with increasing duration. The 4-year incidence of developing proliferative retinopathy in the WESDR younger onset group
increased from 0% during the first 5 years to 27.9% during years 13-14 of diabetes. After 15 years, the incidence of developing PDR remained stable (Fong et al., 2004).

1.4.2.2.2. Pathophysiology

Chronic hyperglycemia, as well as hyperlipidemia and hypertension, contribute to the pathogenesis of diabetic retinopathy (Klein et al., 1998). The vascular disruptions of diabetic retinopathy are characterized by abnormal vascular flow, disruptions in permeability, and/or closure or nonperfusion of capillaries. Endothelial cells are responsible for maintaining the blood-retinal barrier, and damage to them results in increased vascular permeability. Pericytes are essential cellular components in the regulation of retinal capillary perfusion, and damage to these cells in diabetes leads to altered retinal hemodynamics, including abnormal autoregulation of retinal blood flow (Ciulla et al., 2002). Loss of retinal pericytes represents another early feature of diabetic retinopathy (Ansari et al., 1998) and correlates with microaneurysm formation (Speiser et al., 1968). Another common feature of diabetic retinopathy is the thickening of the capillary basement membrane and increased deposition of extracellular matrix components. This feature may contribute to the development of abnormal retinal hemodynamics (Koya and King, 1998), including abnormal autoregulation of retinal blood flow.

1.4.2.2.3. Prevention or Treatment

Current methods of the prevention or treatment of diabetic retinopathy are (i) control of blood glucose, (ii) control of blood pressure, (iii) ablation of pituitary, (iv) retinal laser photocoagulation and (v) vitrectomy (Frank, 2004).
1.4.2.3. Diabetic neuropathy

The neuropathies are among the most common of the long-term complications of diabetes, affecting up to 50% of patients (Cabezas-Cerrato, 1998). Neuropathies are characterized by a progressive loss of nerve fibers, which may affect both principal divisions of the peripheral nervous system (Boulton et al., 2004). They are heterogeneous, affecting different parts of the nervous system that present with diverse clinical manifestations. They may be focal or diffuse. Most common among the neuropathies are chronic sensorimotor distal symmetric polyneuropathy (DPN) and the diabetic autonomic neuropathies (DAN) (Boulton et al., 2005).

1.4.2.3.1. Risk factors

Distal symmetric polyneuropathy (DPN) has been associated with a number of modifiable and nonmodifiable risk factors, including the degree of hyperglycemia, lipid and blood pressure indexes, diabetes duration, and height. DPN has been less consistently associated with cigarette smoking and alcohol consumption. Risk factors for the development of DAN include diabetes duration, age, and long-term poor glycemic control. DAN may cosegregate with factors predisposing to macrovascular events such as raised blood pressure and dyslipidemia (Boulton et al., 2005).

1.4.2.3.2. Pathophysiology

Hypotheses concerning the multiple etiologies of diabetic neuropathy include a metabolic insult to nerve fibers, neurovascular insufficiency, autoimmune damage, and neurohormonal growth factor deficiency. Several different factors have been implicated in this pathogenic process.
Hyperglycemic activation of the polyol pathway leading to accumulation of sorbitol and potential changes in the NAD:NADH ratio may cause direct neuronal damage and/or decreased nerve blood flow. Activation of protein kinase C (PKC) induces vasoconstriction and reduces neuronal blood flow. Increased oxidative stress, with increased free radical production, causes vascular endothelium damage and reduces nitric oxide bioavailability. In a subpopulation of individuals with neuropathy, immune mechanisms may also be involved. Reduction in neurotrophic growth factors, deficiency of essential fatty acids, and formation of advanced glycosylation end products (localized in endoneurial blood vessels) also result in reduced endoneurial blood flow and nerve hypoxia with altered nerve function. The result of this multifactorial process may be activation of polyADP ribosylation, depletion of ATP, resulting in cell necrosis and activation of genes involved in neuronal damage (Vinik et al., 2003).

1.4.2.3.3. Prevention or Treatment

Diabetic patients should maintain aggressive control of blood glucose, HbA1c, blood pressure, and lipids with pharmacological therapy and/or lifestyle changes. All patients with diabetes should be screened for DPN at diagnosis of type 2 diabetes and 5 years after the diagnosis of type 1 diabetes and at least annually by examining sensory function in the feet and checking ankle reflexes. For DAN, screening should be instituted at diagnosis of type 2 diabetes and 5 years after the diagnosis of type 1 diabetes. Screening might comprise a history and an examination for signs of autonomic dysfunction (Boulton et al., 2005).
1.4.2.4. Diabetic foot

Diabetic foot problems are common. Diabetic foot ulcers have a significant impact on quality of life, as well as contributing to disability and premature mortality. Between 40-60% of all amputations are performed in patients with diabetes. A foot ulcer resulting in deep ulceration, uncontrollable infection or gangrene precipitates most amputations. Diabetic foot ulceration is principally associated with peripheral vascular disease and peripheral neuropathy. Previous foot ulceration, amputation, presence of callus, joint deformity, and visual impairment have a cumulative effect on the risk of ulceration (British Medical Association, 2004).

1.4.2.5. Cardiovascular disease

Cardiovascular disease (CVD) is the major cause of morbidity and mortality in patients with type 2 diabetes. Cardiovascular complications are 2 to 6 times greater than microvascular complications and over 60% of all deaths in diabetic patients are due to CVD (Schernthaner, 1996). The risk factors for the development of cardiovascular disease are (i) Hyperglycemia, (ii) Dyslipidemia and (iii) Hypertension (Alberti, 2001).

i) Hyperglycemia

Hyperglycemia might contribute to atherosclerosis in type 2 diabetes in a number of ways. For example, hyperglycemia causes glycosylation of proteins in a process that induces crosslinking of collagen and other extracellular matrix proteins in the arterial wall (Vlassara and Bucala, 1996). The end products of glycation modify low-density lipoprotein (LDL)-cholesterol, prolonging its half-life and producing changes in the artery
rendering it more susceptible to atherosclerosis (Bucala et al., 1993). Among other proposed biochemical pathways in the pathogenesis of diabetic macrovascular disease are glucose-induced activation of PKC isoforms and increased intracellular oxidative stress (Nishikawa et al., 2000).

ii) Dyslipidemia

The central characteristic of dyslipidemia in patients with type 2 diabetes is an elevated triglyceride level, particularly triglyceride-rich very low-density lipoprotein (VLDL) cholesterol levels and decreased high density lipoprotein (HDL) cholesterol levels. In diabetic patients, the concentration of LDL cholesterol is usually not significantly different from that seen in nondiabetic individuals. However, patients with type 2 diabetes typically have a preponderance of smaller, denser, oxidized LDL particles, which may increase atherogenicity (Lamarche et al., 1997) even if the absolute concentration of LDL cholesterol is not elevated (American Diabetes Association, 1998).

This lipid triad, referred to as atherogenic dyslipidemia, is usually present in patients with premature coronary artery disease. When this characteristic lipid profile is seen in type 2 diabetes, it is referred to as diabetic dyslipidemia and confers a risk of CVD that equals or exceeds that of a high-risk LDL cholesterol concentration of 150-220 mg/dl (Grundy, 1997). According to the American Diabetes Association, the presence of increased triglyceride and decreased HDL levels is the best predictor of CVD in patients with type 2 diabetes (American Diabetes Association, 1998).
Other predictive factors include a history of cigarette smoking and hypertension.

iii) Hypertension

In addition to hyperglycemia and lipid abnormalities, which are independently atherogenic, hypertension is also known to be a major risk factor for CVD in all populations (Kannel, 1996). Together, hypertension and overt diabetes substantially and synergistically increase the risk of CVD, as well as of microvascular complications (Deedwania, 2000). Elevated blood pressure is associated with two to three times higher risk of developing congestive heart failure and substantially increases the risk of stroke, with blood pressures of 160 mmHg systolic and/or 90 mmHg diastolic or greater associated with a relative risk of stroke that has been estimated at four times greater than in individuals without hypertension. And in terms of total impact, the role of hypertension is increasing (Eyre et al., 2004).

1.4.2.5.1. Mechanisms of hyperglycemia-induced damage

Four main hypotheses about how hyperglycemia causes diabetic complications are:

(i) increased polyol pathway flux
(ii) increased advanced glycation end-product (AGE) formation
(iii) activation of protein kinase C isoforms
(iv) increased hexosamine pathway flux (Brownlee, 2001).

(i) Increased polyol pathway flux

Aldose reductase is the first enzyme in the polyol pathway that catalyses the NADPH-dependent reduction of a wide variety of carbonyl
compounds including glucose. In a hyperglycemic environment, increased intracellular glucose results in its increased enzymatic conversion to the polyalcohol sorbitol, with concomitant decreases in NADPH. Sorbitol is oxidized to fructose by the enzyme sorbitol dehydrogenase, with NAD\textsuperscript{+} reduced to NADH. The contribution of this pathway to diabetic complications may be very much species, site and tissue dependent (Brownlee, 2001).

(ii) Increased advanced glycation end-product formation

Advanced glycation end products (AGEs) are closely related to hyperglycemia. AGEs are produced when glucose and other reducing sugars react with amino groups in proteins, lipids and nucleic acids. This is a nonenzymatic series of reactions (Maillard reactions) forming Schiff bases and Amadori products. These are then converted to AGEs. Enzymatic activity can be altered by glycosylation and this can produce acute or chronic effects, dependent on the turnover of the enzymes. Glycosylation can also alter the properties of structural proteins with long-term effects (Duckworth, 2001).

Accumulation of AGEs has several toxic effects. AGEs can modify proteins (especially, long-lived proteins such as collagens, lens crystallines and nerve proteins), directly damage the structure and metabolism of extracellular matrix or act via their specific receptors (Bierhaus et al., 1998). In addition to circulation in the blood, AGEs accumulate in tissues and thus take part in the development of diabetic complications. They cause damage to biological membranes and endothelium. Moreover, they modify LDL
particles are together with vascular damage, they are involved in the acceleration of atherosclerosis (Vlassara, 1997).

(iii) Activation of protein kinase C (PKC) isoforms

The PKC family comprises at least eleven isoforms, nine of which are activated by the lipid second messenger diacyl glycerol (DAG). This is achieved primarily by increasing de novo DAG synthesis from the glycolytic intermediate dihydroxyacetone phosphate, through reduction of the latter to glycerol-3-phosphate and stepwise acetylation (Koya and King, 1998). Increased de novo synthesis of DAG activates PKC. Hyperglycemia may also activate PKC isoforms indirectly through both ligation of AGE receptors (Portilla et al., 2000) and increased activity of the polyol pathway (Keogh et al., 1997) presumably by increasing reactive oxygen species.

Abnormal activation of PKC has been implicated in the decreased glomerular production of nitric oxide induced by experimental diabetes (Craven et al., 1994) and in the decreased production of nitric oxide in smooth muscle cells that is induced by hyperglycemia (Ganz and Seftel, 2000). Activation of PKC contributes to increased microvascular matrix protein accumulation by inducing expression of tumor growth factor (TGF)-β1, fibronectin and type IV collagen both in cultured mesangial cells (Studer et al., 1997) and in glomeruli of diabetic rats (Koya et al., 1997).

(iv) Increased hexosamine pathway flux

Shunting of excess intracellular glucose into the hexosamine pathway might also cause several manifestations of diabetic complications (Kolm-Litty et al., 1998). In this pathway, fructose 6-phosphate is diverted from
glycolysis to provide substrates for reactions that require UDP-N-acetylglicosamine, such as proteoglycan synthesis and the formation of O-linked glycoproteins. Inhibition of the rate-limiting enzyme in the conversion of glucose to glucosamine-glutamine:fructose 6-phosphate amidotransferase blocks hyperglycemia-induced increases in the transcription of TGF-α, TGF-β1 (Kolm-Litty et al., 1998) and plasminogen activator inhibitor (PAI-1) (Du et al., 2000). The activation of the hexosamine pathway by hyperglycemia may result in many changes in both gene expression and protein function, which together contribute to the pathogenesis of diabetic complications (Brownlee, 2001).

1.5. Abnormalities in diabetes mellitus

Type 2 diabetes presents as a spectrum of metabolic abnormalities with prominent insulin resistance and relative insulin deficiency (American Diabetes Association, 2006). The effect of diabetes is not limited to carbohydrate metabolism. Lipid and protein metabolism also play an important role in the progression of the disease (Uusitupa et al., 1993). Insulin is essential for maintaining glucose homeostasis and regulating carbohydrate, lipid, and protein metabolism (Saltiel and Kahn, 2001).

1.5.1. Abnormalities of carbohydrate metabolism

Alterations in glucose metabolism in diabetes are frequently accompanied by changes in the activities of the enzymes that control glycolysis and gluconeogenesis in liver and muscle, such that the latter process becomes favoured (Gerich, 1993). Insulin suppresses hepatic glucose output by stimulating glycogen synthesis and inhibiting
glycogenolysis and gluconeogenesis. Increased rate of hepatic glucose production result in the development of overt hyperglycemia, especially fasting hyperglycemia, in patients with type 2 diabetes (DeFronzo et al., 1992). Insulin exerts direct effect on the liver (Michael et al., 2000) as well as influences the substrate availability and fluxes of free fatty acids (FFA) (Bergman and Ader, 2000). There are several important enzymatic checkpoints that act to control hepatic glycolysis and glycogen synthesis (glucokinase, glycogen synthase kinase-3), glycogenolysis (phosphorylase), gluconeogenesis (phosphoenolpyruvate carboxykinase, fructose 1,6-bisphosphatase), or steps that are common to the pathways (glucose 6-phosphatase). Some of them are directly controlled by insulin via phosphorylation and dephosphorylation (Zhang, 2002).

1.5.2. Abnormalities of lipid metabolism

In patients with type 1 diabetes in good glycemic control, the lipid profile is very similar to lipid profiles in the general population. In contrast, in patients with type 2 diabetes, even when in good glycemic control, there are abnormalities in lipid levels. Specifically, patients with type 2 diabetes often have an increase in serum triglyceride (TG) levels, increased VLDL and an intermediate density lipoprotein (IDL), decreased HDL, and an increase in small dense LDL, a lipoprotein particle that may be particularly atherogenic. In both type 1 and type 2 diabetes, poor glycemic control increases serum TG levels, VLDL and IDL, and decreases HDL. Poor glycemic control can also result in a modest increase in LDL cholesterol, which because of the elevation in TG is often in the small dense subfraction. It is therefore,
important to optimize glycemic control in patients with diabetes because this will have secondary beneficial effects on lipid levels. Lipoprotein (Lp) (a) levels are usually within the normal range in patients with type 2 diabetes and do not appear to be greatly affected by glycemic control. In patients with type 1 diabetes, Lp (a) levels are frequently elevated and improvements in glycemic control result in decreases in Lp (a) levels. The development of microalbuminuria and the onset of renal disease are associated with an increase in Lp (a) levels (Feingold, 2004).

1.5.3. Abnormalities of protein metabolism

Diabetes mellitus is basically a disorder of carbohydrate metabolism, but with progression of the disease, protein metabolism is also affected (Khan and Safdar, 2003). An association between diabetes mellitus and protein catabolism has been well documented. Many of the chronic complications of diabetes involve changes in structural proteins. It is thus possible that changes in protein metabolism are responsible for many of the chronic complications of diabetes mellitus, because even a minor imbalance between protein synthesis and degradation can potentially have a profound effect over the long term on cell viability and metabolism. Alterations in protein synthesis and degradation can also adversely affect the repair of tissue after injury or infection (Charlton and Nair, 1998).

1.5.4. Free radicals

Reactive oxygen species (ROS) is a term, which encompasses all highly reactive, oxygen-containing molecules, including free radicals. Types of ROS include the hydroxyl radical, the superoxide anion radical, hydrogen
peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical and various lipid peroxides. All are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes, and other small molecules, resulting in cellular damage.

Oxygen free radicals or, more generally, ROS as well as reactive nitrogen species (RNS), are products of normal cellular metabolism. ROS and RNS are well recognised for playing a dual role as both deleterious and beneficial species, since they can be either harmful or beneficial to living systems (Valko et al., 2006). The harmful effect of free radicals causing potential biological damage is termed oxidative stress (Ridnour et al., 2005). This occurs in biological systems when there is an overproduction of ROS/RNS on one side and a deficiency of enzymatic and non-enzymatic antioxidants on the other. The excess ROS can damage cellular lipids, proteins, or DNA inhibiting their normal function. Increased oxidative stress has been proposed to be one of the major causes of the hyperglycemia-induced trigger of diabetic complications. Hyperglycemia in an organism stimulates ROS formation from a variety of sources. These sources include oxidative phosphorylation, glucose autoxidation, NAD(P)H oxidase, lipoxygenase, cytochrome P450 monoxygenases and nitric oxide synthase (NOS).

Oxygen-derived free radicals (OFRs) have been implicated in the pathophysiology of various disease states, including diabetes mellitus (Giugliano et al., 1996). It is well known that superoxide anion ($O_2^-$) is the primary radical formed by the reduction of molecular oxygen that may lead to
secondary radicals or ROS such as hydrogen peroxide (H₂O₂), and hydroxyl radical (•OH) (Katusic, 1996). The effects of OFRs on the vascular systems and their mechanism(s) of action are not clear. In addition to its action as an oxidant, H₂O₂ can elicit different and sometimes complex actions on the tone of blood vessel (Rubanyi, 1987).

1.5.4.1. Lipid peroxidation

Lipid peroxidation is a free-radical mediated propagation of oxidative insult to polyunsaturated fatty acids (PUFA) involving several types of free radicals and termination occurs through enzymatic means or by free radical scavenging by antioxidants (Korkina and Afanas'ev, 1997). When free radicals and other reactive species (e.g., •OH, HOO•, ONOO−) extract a hydrogen atom from an unsaturated fatty acyl chain (e.g., ω-6 polyunsaturated fatty acid), a carbon-centered lipid radical (L•) is produced. This is followed by the addition of oxygen to L• to yield a lipid peroxyl radical (LOO•). LOO• further propagates the peroxidation chain reaction by abstracting a hydrogen atom from a nearby unsaturated fatty acid. The resulting lipid hydroperoxide (LOOH) can easily decompose to form a lipid alkoxyl radical (LO•).

This series of ROS-initiated lipid peroxidation reactions with the production of lipid peroxyl and alkoxyl radicals, collectively called chain propagation, occurs in mammalian cells, such that oxygen free radicals may cause damage far in excess of their initial reaction products (Fang et al., 2002).

Initiation: LH + •OH → H₂O + L•
1.5.4.2. Oxidative stress

'Oxidative stress' is a condition where the generation of free radicals exceed the scavenging abilities of endogenous antioxidant defenses (Ruhe and McDonald, 2001). In both diabetic patients and experimental models of diabetes mellitus, markers of increased oxidative stress have been identified in blood and tissues (Baynes and Thorpe, 1999).

1.5.4.2.1. Oxidative stress and diabetes

Oxidative stress is playing a major role in the pathogenesis of both types of diabetes mellitus. Free radicals are formed in diabetes by glucose oxidation, protein glycation and the subsequent degradation of glycated proteins. High levels of free radicals and the simultaneously declined antioxidant enzyme levels lead to cell damage, inactivation of enzymes and lipid peroxidation. Accumulated evidence also indicates that oxidative stress-activated signaling pathways mediate insulin resistance and β-cell dysfunction. These consequences of oxidative stress can promote the development of diabetes complications.

Alterations in the antioxidant enzyme activities and increased oxidative damage have been demonstrated in different tissues of diabetic animals (Kakkar et al., 1995). Therefore, increased oxidative stress in
diabetes leads to alterations of cellular redox status, which should be regarded as the pivotal role in diabetes and its complications.

1.5.4.3. Antioxidants

Antioxidants are the first line of defense against free radical damage and are critical for maintaining the optimum health and wellbeing (Mark, 1998). Normal cells are protected against the deleterious effect of ROS by antioxidant defense mechanisms. An "antioxidant is any substance that when present in low concentration compared to those of the oxidizable substrate significantly delays or prevents oxidation of that substrate" (Ruiz et al., 1999). Antioxidants can be divided into three groups: (a) metal chelators, such as transferrin and ceruloplasmin, which inhibit the initiation phase of oxygenated free radical production, (b) free radical scavengers, such as vitamin E, vitamin C, reduced glutathione (GSH), uric acid, bilirubin and serum albumin, which act on the propagation phase of lipid peroxidation, (c) antioxidant enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) and catalase (Bonnefont et al., 2000).

1.5.4.3.1. Antioxidants and diabetes

Oxidative stress results from an imbalance between radical generating and radical scavenging systems (i.e.) increased free radical production or reduced activity of antioxidant defenses or both of these phenomena. There are several lines of evidence to suggest that antioxidant defences may be lower in diabetes. These include reports of reduced plasma/serum total antioxidant status or free radical scavenging activity and increased plasma oxidisability in type 2 diabetics, together with
demonstrations of reduced levels of specific antioxidants such as ascorbic acid and vitamin E (Ashour et al., 1999). In addition, the activities of the antioxidant enzymes catalase, SOD and GPx, have been described as reduced in diabetics (Ashour et al., 1999). A diminution in the endothelial synthesis of Nitric Oxide has also been suggested in type 2 diabetics (Makimattila et al., 1999), which apart from detracting from vascular antioxidant defense would of course compound any defect in the antiatherogenic signalling role of Nitric Oxide (Laight et al., 1999). Recent results suggest a potential usefulness of antioxidants for treating diabetes and provides further support for the implication of oxidative stress in β-cell dysfunction in diabetes (Valko et al., 2006).

1.6. Non pharmacological therapy

Diet, exercise and weight loss are at the center of any therapeutic programme. Not only do these lifestyle modifications which reduce the blood glucose concentrations, but also they ameliorate many of the frequently coexisting risk factors for CVD. Most of the patients are unable to achieve adequate control with lifestyle interventions alone. Therefore, a controlled-energy diet and regular aerobic exercise are recommended for the majority of patients with type 2 diabetes who are usually overweight (American Diabetes Association, 1997).

1.7. Pharmacological therapy

1.7.1. Sulphonylureas

The first generation sulphonylureas (SUs) are chlorpropamide, tolbutamide, acetohexamide and tolazamide. The second generation SUs
include glyburide, glipizide and glimepiride and are more potent than the first generation SUs (Cohen and Harris, 1987). The SUs bind to the SU receptor, found on the surface of pancreatic β-cells. This interaction leads to a closure of voltage-dependent potassium adenosinetriphosphate (K\textsubscript{ATP}) channels, facilitating cell membrane depolarization, calcium entry into the cell and insulin secretion (Zimmerman, 1997). Thus, SUs allow for insulin release at lower glucose thresholds than normal and the circulating insulin concentrations are increased.

Of more practical concern, SU therapy is associated with two common adverse effects. The first is weight gain and the second is hypoglycemia, most likely to affect the elderly, those with worsening renal function and those with irregular meal schedules (Zimmerman, 1997). SUs are approved for use as monotherapy and in combination with all other oral agent classes (except the non-SU secretagogues) and insulin (Inzucchi, 2002).

1.7.2. Biguanides

Metformin and phenformin were the two main biguanides that were introduced in the late 1950s (Beckman, 1971). Phenformin was withdrawn from clinical use in many countries in the late 1970s when an association with lactic acidosis was recognized (Nattrass and Alberti, 1978). Metformin is now used in more than 90 countries. Metformin does not stimulate insulin secretion (Johansen, 1999) and its predominant effect is to reduce hepatic glucose production in the presence of insulin (Hundal \textit{et al.}, 2000).
Metformin monotherapy is associated with weight loss (or no weight gain) and much less hypoglycemia than SU therapy (Johansen, 1999). Other nonglycemic benefits have also been ascribed to metformin, such as decreases in lipid levels (LDL cholesterol and TG) and the antifibrinolytic factor PAI-1 (Fontbonne et al., 1996). Adverse effects of metformin therapy include gastrointestinal distress such as abdominal pain, nausea, and diarrhea (Bailey and Turner, 1996). It is approved for use as monotherapy and in combination with SUs and other secretagogues, thiazolidinediones (TZDs) and insulin (Inzucchi, 2002).

1.7.3. α-glucosidase inhibitors

The α-glucosidase inhibitors (AGIs; acarbose and miglitol) were introduced in 1996. Their mechanism of action is unique and this is the sole drug class not targeted at a specific pathophysiological defect of type 2 diabetes mellitus. An enzyme in the brush border of the proximal small intestinal epithelium, α-glucosidase serves to break down disaccharides and more complex carbohydrates. By the competitive inhibition of the enzyme, the AGIs delay intestinal carbohydrate absorption and mitigate postprandial glucose excursions (Lebowitz, 1998).

The efficacy of AGIs is considerably less than that of either SUs or metformin. The AGIs are attractive in that they are essentially nonsystemic and unassociated with hypoglycemia and weight gain. Nonglycemic benefits include small reductions in TG and postprandial insulin levels (Lebowitz, 1998). Adverse effects of AGIs include flatulence, abdominal discomfort and
diarrhea. They are approved for use as monotherapy and in combination with SUs (lnzucchi, 2002).

1.7.4. Thiazolidinediones

Thiazolidinediones, currently represented by rosiglitazone and pioglitazone has a unique mechanism of action. They are pharmacological ligands for a nuclear receptor known as peroxisome-proliferator-activated receptor gamma (PPARγ). When activated, the receptor binds with response elements on DNA, altering transcription of a variety of genes that regulate carbohydrate and lipid metabolism (Mudaliar and Henry, 2001). The most prominent effect of TZDs is increased insulin-stimulated glucose uptake by skeletal muscle cells (Frias et al., 2000). Hepatic glucose production is decreased although perhaps only at the highest doses (Yu et al., 1999). PPARγ activation also reduces lipolysis and enhances adipocyte differentiation (Chao et al., 2000).

In addition to their ability to lower insulin levels, the TZDs also have certain lipid benefits. HDL cholesterol concentrations increase with TZD therapy and TG concentrations frequently fall (Horton et al., 1998). Adverse effects of TZDs include weight gain, which can be as great or greater than that with the SUs (Kelly et al., 1999). TZDs are the most expensive class of antidiabetic medication and are indicated as monotherapy and in combination with metformin, SUs and insulin (lnzucchi, 2002).

1.7.5. Non-SU secretagogues

Repaglinide and nateglinide are the non-SU secretagogues. The mechanism of action of these drugs is similar to that of SUs. They are
distinguished from the SU's by their short metabolic half-lives, which result in brief episodic stimulation of insulin secretion (Perfetti and Ahmad, 2000). There are two important consequences from this difference. First, postprandial glucose excursions are attenuated because of greater insulin secretion immediately after the ingestion of meal (Hirschberg et al., 2000). Second, because less insulin is secreted several hours after the meal, there is decreased risk of hypoglycemia during this late postprandial phase (Nattrass and Lautrizen, 2000).

Adverse effects include hypoglycemia and weight gain, which are probably less pronounced than that caused by SU's (Marbury et al., 1999). One disadvantage of this drug category is the frequent dosing schedule required with meals. They are approved for use either as monotherapy or in combination with metformin (Inzucchi, 2002).

**Figure 1. Causes of hyperglycemia and action of oral antidiabetic drugs** (Florence and Yeager, 1999).
1.8. Streptozotocin (STZ)

Streptozotocin (STZ, CAS No. 18883-66-4) is a monofunctional nitrosurea derivative that was first isolated from *Streptomyces achromogenes* fermentation broth (Herr *et al.*, 1967). Its molecular structure corresponds to a 2-deoxy-D-glucose molecule substituted at C2 with a N-methyl-N-nitrosurea group (Herr *et al.*, 1967). STZ is a broad spectrum antibiotic and is often used to induce diabetes mellitus in experimental animals through its toxic effects on pancreatic β-cells (Junod *et al.*, 1967).

![Figure 2. Structure of streptozotocin (Herr *et al.*, 1967)](image)

STZ is a potent alkylating agent known to directly methylate DNA (Tjalve, 1983) and induces DNA damage by methylation of guanines via methyl cations (Bozlan and Bianchi, 2002). A single i.v. dose of STZ (50-100 mg/kg) induces strand breaks in liver, kidney, and pancreatic islets of rats (Yamamoto *et al.*, 1981). Free radicals play an essential role in the mechanism of DNA damage and cytotoxicity by STZ. It enhances $O_2^+$ radical
generation by the xanthine oxidase system of pancreatic \( \beta \)-cells (Kawada, 1992) and stimulates \( \text{H}_2\text{O}_2 \) generation and causes DNA fragmentation in isolated rat pancreatic islets (Takasu et al., 1991).

DNA lesions produced by STZ includes double and single-strand breaks, covalent adducts and alkali-labile sites. Severe DNA damage by STZ results in cell death by apoptosis or necrosis. The DNA strand breaks resulting from the alkylating action of STZ can lead to chromosomal rearrangements (Bozlan and Bianchi, 2002). Nitric oxide generation during cellular metabolism of STZ contributes to rat pancreatic islet cell DNA damage (Kroncke et al., 1995).

STZ action in \( \beta \)-cells is accompanied by characteristic alterations in blood insulin and glucose concentrations. Two hours after injection, the hyperglycemia is observed with a concomitant drop in blood insulin. About six hours later, hypoglycemia occurs with high levels of blood insulin. Finally, hyperglycemia develops and blood insulin levels decrease (West et al., 1996). STZ is taken up by pancreatic \( \beta \)-cells via glucose transporter 2 (GLUT2). A reduced expression of GLUT2 has been found to prevent the diabetogenic action of STZ (Thulesen et al., 1997).

1.9. Flavonoids

Flavonoids represent the most common and widely distributed group of plant phenolics and are abundant in foods. Over 4000 structurally unique flavonoids have been identified in plant sources (Harborne, 1986) and found in fruits, vegetables, nuts, seeds, herbs, spices, stems, flowers as well as tea and red wine. Many of the flavonoids are responsible for the attractive
colours of flowers, fruits and leaves. They are defined as non-nutritive dietary components (Boyle et al., 2000) and are prominent components of citrus fruits and other food sources (Herrmann, 1976). They are consumed regularly with the human diet. Flavonoids appear to be remarkably safe and adverse reactions to flavonoids in humans appears to be rare.

Flavonoids display a remarkable array of biochemical and pharmacological actions, some of which suggest that certain members of this group of compounds may significantly affect the function of various mammalian cellular systems. They possess several physiological properties: antioxidant, antibacterial, antiviral, anti-inflammatory, anti-mutagenic, anticancer and activation or inactivation of certain enzymes (Rice-Evans and Packer, 1998). Epidemiological studies have shown a negative correlation between flavonoid intake and the occurrence of CVD (Knet et al., 1996). Regular ingestion of flavonoid-containing foods may protect against death from coronary artery disease in elderly men (Hertog et al., 1993).

1.9.1. Quercitrin

Among flavonoids, quercetin is the most common flavonoid in nature, and it is mainly present as its glycosylated forms such as quercitrin (5,7,3',4'-OH, 3-rhamnosylquercetin) (Figure 3) (Hertog et al., 1993). A wide variety of pharmacological activities of quercitrin was reported viz. anti-inflammatory (Taguchi et al., 1993; Sanchez de Medina et al., 2002), antidiarrhoeal (Galvez et al., 1993), anti-inflammatory property (Gadotti et al., 2005), antileishmanial activity (Muzitano et al., 2006) and neuroprotective (Hollman and Katan, 1999). However, the majority of the studies have been
carried out with the aglycone (quercitin) form and little is known about the biological properties of glycoside forms, due to the lack of commercial standards.

Figure 3. Structure of Quercitrin

1.9.1.1. Pharmacokinetics

Quercitrin can be absorbed intact from the intestine and is detected as such in the plasma. However, the possibility that it was hydrolyzed to the aglycone quercetin in the liver (Walle, 2004) and in the intestine by bacterial rhamnosidases (Park et al., 2005) cannot be ruled out. In this context, it has been demonstrated in an experimental model of rat colitis that quercitrin releases quercetin after glycoside's cleavage in the intestine, in order to perform its anti-inflammatory effect which is mediated through the inhibition of the NF-kappaB pathway (Comalada et al., 2005).