Introduction/

Review of Literature
The prevalence of diabetes for all age groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of subjects with diabetes is projected to increase from 171 million in 2000 to 366 million in 2030 (WHO, 2006). The prevalence of diabetes is higher in men than women. The most important demographic change to diabetes prevalence across the world appears to be the increase in the proportion of people greater than 65 years of age (Wild et al, 2004). According to recent estimates, close to 77% of the global burden of disease is projected to occur in the developing countries. The increasing prevalence of diabetes in developing countries is closely associated with industrialization and socioeconomic development. The major determinants for projected increase in the number of diabetics in these countries are: population growth; age structure, and urbanization. The urban population in developing countries is projected to double between 2000 and 2030. With the rise in the urban/rural population ratio in all regions, and growing prevalence of obesity among urban dwellers, diabetes will increasingly concentrate in the urban areas. Important differences are observed in age structure of diabetic population between developed and developing countries. In the developed countries, the majority of diabetics are aged 65 years and above, whereas it was 45-64 years in the developing countries. This means that in developing countries, the majority of diabetic patients acquire the condition during the most productive period of their lives. This will have major implications with respect to health care needs.

Diabetes mellitus, once thought to be uncommon in the developing world, has now emerged as an important public health problem in Asia. An estimated 47 million individuals in the South-East Asia Region (SEAR) are affected at present. It is estimated that by the year 2030 there will be 119.5 million diabetics in the Region-the highest among all WHO regions. Thus the SEAR will bare the maximum global burden of the disease (WHO, 2006). A bulk of evidence from studies on migrants indicates that the ethnic, presumably genetic vulnerability of Asians manifests in to diabetes when subjected to unfavorable life styles. The population in India has an increased susceptibility to diabetes mellitus. This propensity was demonstrated by multiple surveys of migrant Indians residing in Fiji, Singapore, South Africa, UK, and USA. The occurrence of diabetes in migrants from the Indian subcontinent has consistently shown to exceed those of the local population. The results of prevalence
studies of diabetes mellitus in India were systematically reviewed with emphasis on those utilizing the standard WHO criteria for diabetes diagnosis. In the year 2000 an estimated 31.7 million individuals in India were reported to be diabetics and it is projected that by the year 2030 this number will be increased to 79.4 million. Since 1995, India has been ranked number one with the highest number of cases of diabetes, followed by China, and USA (Wild et al., 2004). The American Heart Association has identified diabetes mellitus as an independent major risk factor for cardiovascular disease (CVD) in both men and women, which is the most costly complication (Grundy et al., 1999; Brown et al., 1999). The cardiovascular complications of diabetes mellitus include coronary heart disease (CHD), stroke, peripheral arterial disease, nephropathy, retinopathy, and possibly neuropathy including cardiomyopathy. Because of the aging of the population and an increasing prevalence of obesity and sedentary life style in the developed as well as developing countries, the prevalence of diabetes is increasing. In fact, from the point of view of cardiovascular medicine, diabetes may be termed as a cardiovascular disease (Grundy et al., 1999). According to a WHO report (2006), it was estimated that during 2000 about 171 million persons worldwide were affected by diabetes mellitus. Out of which, 5 to 10 % were designated as type 1 (formerly known as insulin-dependent) and 90 to 95 % were type 2 (non-insulin-dependent) diabetes mellitus. Type 1 diabetes is characterized by an absolute deficiency of insulin caused by immunologic destruction of pancreatic β-cells (Unger and Foster, 1998). Type 1 diabetes usually begins early in life and is often called juvenile diabetes. This form of diabetes frequently produces microvascular complications, nephropathy and retinopathy (Unger and Foster, 1998), but it also predisposes to CHD (Lloyd et al., 1996). The metabolic abnormalities of diabetes mellitus result from a deficiency of insulin and a relative excess of glucagon. These imbalances in hormonal levels most profoundly affect various metabolic pathways in liver, muscle, adipose tissue (Fig. 1.1, Champe, et al., 2005). Elevated levels of blood glucose and ketone bodies are the hallmarks of untreated diabetes mellitus.

Hyperglycemia is caused by increased hepatic production of glucose in conjunction with diminished peripheral use due to an inability of glucose uptake by muscle and adipose cells. Ketosis results from substantial mobilization of fatty acids
Fig. 1.1. Intertissue relationship in type 2 diabetes.

from adipose tissue, combined with accelerated hepatic ketogenesis. Diabetic ketoacidosis occurs in 25 to 40% of newly diagnosed type 1 diabetes, and may recur if the patient becomes ill (most commonly with an infection) or does not comply with therapy. Ketoacidosis is treated by replacing fluids and electrolytes, followed by low-dose insulin administration to gradually correct hyperglycemia. The excess liver fatty acids left behind, after their oxidation or conversion to ketone bodies, are converted to triglyceride (TG), which is packaged and secreted in very low density lipoprotein (VLDL). Since in diabetes due to low lipoprotein lipase activity in adipose tissue, degradation of chylomicrons is reduced, the plasma chylomicrons and VLDL levels are increased resulting in hypertriglyceridemia. Patients with type 1 diabetes have virtually no functional β-cells, and can neither respond to variations in circulating fuels nor maintain a basal secretion of insulin. Therefore, this type of diabetic must rely on exogenous insulin administered subcutaneously to control the hyperglycemia and ketoacidosis. The most prevalent form of diabetes mellitus is type 2 diabetes, which is heterogeneous disorder. This disorder typically makes its appearance later in life and its clinical expression requires both genetic and environmental factors. The underlying metabolic causes of type 2 diabetes are the combination of impairment in insulin-mediated glucose disposal (insulin resistance) and defective secretion of insulin by pancreatic β-cells. Insulin resistance develops from obesity and physical inactivity, acting on a substrate of genetic susceptibility (Gerick, 1998; Chisholm et al., 1997). Insulin secretion declines with advancing age (Muller et al., 1996; Dechenes et al., 1998), and this decline may be accelerated by genetic factors (Pimenta et al. 1995; Humphriss et al., 1997). Insulin resistance typically precedes the onset of type 2 diabetes and is commonly accompanied by other cardiovascular risk factors, such as, dyslipidemia, hypertension and prothrombotic factors (Hopkins et al., 1996; Gray et al., 1998). The common clustering of these risk factors in an individual has been called the metabolic syndrome. Many patients with the metabolic syndrome manifest impaired fasting glucose (Unger and Foster, 1998) even when they do not have overt diabetes mellitus (Stern, 1996). The metabolic syndrome commonly precedes the development of type 2 diabetes by many years (Haffner et al., 1990). It is important to mention that the risk factors that constitute this syndrome, contribute independently to CVD risk.
Recently, new criteria have been accepted for the diagnosis of diabetes (ECDCDM, 1997). The upper threshold of fasting plasma glucose for the diagnosis of diabetes has been lowered from ≥ 140 mg/dl to ≥ 126 mg/dl. The upper threshold for normoglycemia has been reduced from < 115 to 125 mg/dl is now designated as impaired fasting glucose. Patients with type 2 diabetes have a combination of insulin resistance and dysfunctional β-cells, but do not require insulin to sustain life, although insulin may be required to control hyperglycemia in some patients. The occurrence of the type 2 diabetes is almost completely determined by genetic factor. Diagnosis is based on the presence of hyperglycemia—that is, a blood glucose concentration of greater than 126 mg/dl. Insulin resistance is the decrease ability of target tissues, such as liver, adipose, and muscle, to respond properly to normal circulating concentrations of insulin. For example, insulin resistance is characterized by uncontrolled hepatic glucose production, and decreased glucose uptake by muscle and adipose tissue. Obesity is the most common cause of insulin resistance. Most people with obesity and insulin resistance do not become diabetic. In the absence of a defect in β-cell function, nondiabetic, obese individuals can compensate for insulin resistance with elevated levels of insulin. For example, insulin secretion is 2 to 3 times higher in obese subjects in comparison to lean individuals. This higher insulin level compensates for the diminished effect of the hormone (as a result of insulin resistance), and produces blood glucose levels similar to those observed in lean individuals. Insulin resistance alone will not lead to type 2 diabetes. Rather, type 2 diabetes develops in insulin-resistant individuals who also exhibit impaired β-cell function. Insulin resistance and subsequent development of type 2 diabetes is commonly observed in the elderly, and in individuals who are obese, physically inactive, or in women who are pregnant. These patients are unable to sufficiently compensates for insulin resistance with increased insulin release. Insulin resistance increases with body weight gain, and conversely, diminishes with weight loss. This suggests that fat accumulation is important in the development of insulin resistance. Regulatory substances, such as leptin, resistin and adiponectin produced by adipocytes may contribute to the development of insulin resistance. In addition, the elevated levels of free fatty acids in obesity have also been implicated in the development of insulin resistance. In type 2 diabetes, the β-cell is dysfunctional because it fails to secrete enough insulin to correct the prevailing hyperglycemia. Thus, the natural progression of the disease results in a
declining ability to control hyperglycemia with endogenous secretion of insulin. Deterioration of β-cell function may also be accelerated by the toxic effects of sustained hyperglycemia and elevated levels of free fatty acid. The metabolic abnormalities of type 2 diabetes mellitus are the result of insulin resistance expressed primarily in liver, muscle and adipose tissue, summarized in Fig. 1.2. Hyperglycemia is caused by increased production of glucose combined with decreased peripheral use. Ketosis is usually minimal or absent in type 2 diabetic patients because the presence of insulin. In the liver, fatty acids are converted to TGs, which are packaged and secreted in VLDLs. Chylomicrons are synthesized from dietary lipids by the intestinal mucosal cells following a meal. Because of low lipoprotein lipase activity in adipose tissue, lipoprotein degradation is diminished in diabetics. Therefore, in type 2 diabetic patients with the plasma chylomicron and VLDL levels remain elevated, resulting in hypertriglyceridemia.

As already mentioned, clustering of metabolic risk factors, called the metabolic syndrome, occurs commonly in type 2 diabetes (ADA, 1989). The onset of hyperglycemia in patients with the metabolic syndrome appears to accelerate atherogenesis, possibly by enhanced formation of glycosylated proteins and advanced glycation products (Brownlee et al., 1988: Hammers and Brownlee, 1996) and/or by increasing endothelial dysfunction (Nadler and Winer, 1996). These direct consequences of hyperglycemia probably contribute to the microvascular disease, underlying, retinopathy, nephropathy, and neuropathy, and they may promote macrovascular disease as well. The individual with diabetes has a 25-fold increase in the risk of blindness a 20-fold increase in the risk of renal failure, and a 20-fold increase in the risk of amputation as a result of gangrene (Klein et al., 1985). How hyperglycemia causes the chronic complications of diabetes is unclear. In cells where entry of glucose is not dependent on insulin, elevated blood glucose leads to increased intracellular glucose and its metabolites. For example, increased intracellular sorbitol contributes the formation of cataract. In addition, hyperglycemia promotes the condensation of glucose with cellular proteins in a reaction analogous to the formation of HbA1. These glycated proteins mediate some of the early microvascular changes of diabetes. Glycosylation of retinal proteins and retinal microvascular abnormalities leads to retinopathy and eventually blindness (Kelvin and Moss, 1992). In addition, a
Fig. 1.2. Typical progression of type 2 diabetes.
sustained high level of blood and tissue glucose is also responsible for the development of diabetic nephropathy, which is characterized by thickening of the basement membranes in renal glomeruli and peripheral capillaries (nodular glomerulosclerosis) as observed in diabetic patients (Anderson et al., 1983), and in STZ-induced diabetic rats (Olgemoller and Schleicher, 1993). Diabetic nephropathy is the most common cause of chronic kidney failure and end-stage kidney disease in the USA. People with both type 1 and type 2 diabetes are at risk and the disease may cause death 2 or 3 years after the initial lesion formation (AHSMD, 1995). Most patients with type 2 diabetes have insulin resistance, which seems to predispose to both CVD and diabetes (Reaven, 1996). Several lines of research suggest that insulin resistance is a multisystem disorder that induces multiple metabolic alterations. Metabolic risk factors that commonly occur in patients with insulin resistance are glucose intolerance, hypertension, prothrombotic state, and atherogenic dyslipidemia (Reaven, 1996). Hypertension is a well-established major risk factor for CVD (Wilson, 1998). It increases the risk for both CHD and stroke and contributes to diabetic nephropathy (Nelson et al., 1993). Several investigators (Edelson and Sowers, 1993; Sowers, 1990) report a positive association between insulin resistance and hypertension, which suggests that elevated blood pressure, deserves to be listed among the components of the metabolic syndrome. Hypertension nonetheless is a multifactorial disorder, and the mechanistic connections between insulin resistance and hypertension are largely conjectural. However, evidence for a casual link is growing (Reaven et al., 1996). When hypertension coexists with overt diabetes the risk for CVD including nephropathy is doubly increased. The first abnormality in plasma glucose in patients with insulin resistance is impaired fasting glucose tolerance (IFG), (ECDCDM, 1997). The presence of IFG usually accompanies long-standing insulin resistance. Many prospective studies (Haffner, 1997; Laakso and Letho, 1998) show that IFG is a risk factor for CVD; the degree of independence as a risk factor, however, is uncertain, because IFG usually coexist with other components of the metabolic syndrome (Haffner et al., 1990). Nonetheless, a patient with IFG must be considered at risk for both CVD and type 2 diabetes. As indicated above, once categorical hyperglycemia develops, it is considered as an independent risk factor for CVD (Wilson, 1998). A newly recognized component of the metabolic
syndrome is a prothrombotic state (Reaven et al., 1996). Patients with insulin resistance frequently manifest several alterations in coagulation mechanism that predispose them to arterial thrombosis. These alterations include increased fibrinogen levels (Imperatore et al., 1998), increased plasminogen activator inhibitor-1 (Byberg et al., 1998), and various platelet abnormalities (Trovati and Anfossi, 1998). Diabetes mellitus substantially increases the risk of developing coronary, cerebrovascular and peripheral arterial disease. The pathophysiology of vascular disease in diabetes involves abnormalities in endothelial, vascular smooth muscle cell, and platelet function. The metabolic abnormalities that characterized diabetes, such as hyperglycemia, increased free fatty acids, and insulin resistance, each provoke molecular mechanisms that contribute to vascular dysfunction. These include decreased bioavailability of nitric oxide (NO), increased oxidative stress, disturbances of intracellular signal transduction and activation of receptors of AGEs. In addition, platelet function is abnormal, and there is increased production of several prothrombotic factors. These abnormalities contribute to the cellular events that cause atherosclerosis and subsequently increase the risk of the adverse cardiovascular events that occur in patients with diabetes and atherosclerosis (Creager et al., 2003). A better understanding of the mechanisms leading to vascular dysfunction may unmask new strategies to reduce cardiovascular morbidity and mortality in patients with diabetes.

1.1 Pathogenesis of Diabetic Complications

The Diabetes Control and Complications Trial (DCCT) and the UKPDS (U. K. Prospective Diabetes Study), established that hyperglycemia, is the initiating cause of the diabetic tissue damage that we see clinically (DCCTRG, 1993; UKPDS, 1998). Although this process is modified by both genetic determinants of individual susceptibility and by independent accelerating factors such as hypertension and hyperlipidemia. The tissue damaging effects of hyperglycemia is restricted to a particular subset of cell types: capillary endothelial cells in the retina, mesangial cells in the renal glomerulus, and neurons as well as Schawan cells in peripheral nerves. It is well known that in diabetes, hyperglycemia is bathing all the cells of every tissue. However, most cells are able to reduce the transport of glucose inside the cell when they are exposed to hyperglycemia, so that their internal glucose concentration stays constant. In contrast, the cells damaged by hyperglycemia are those that can not do
this efficiently (Kaiser et al., 1993; Heilig et al., 1995). Thus, diabetes selectively damages cells, like endothelial cells and mesangial cells, whose glucose transport rate does not decline rapidly as a result of hyperglycemia, leading to high glucose inside the cell.

The first such mechanism that was discovered was the polyol pathway and increased polyol pathway flux, described in peripheral nerve (Gabbay et al., 1966). The polyol pathway, shown schematically in Fig. 1.3, focuses on the enzyme aldose reductase. Aldose reductase normally has the function of reducing toxic aldehydes in the cell to inactive alcohols, but when the glucose concentration in the cell becomes too high, aldose reductase also reduces that glucose to sorbitol, which is latter oxidized to fructose. In the process of reducing high intracellular glucose to sorbitol, the aldose reductase consumes the cofactor NADPH (Lee and Chung, 1999). But as shown in Figure 1.3, NADPH is also the essential cofactor for regenerating a critical intracellular antioxidant, reduced glutathione. By reducing the amount of reduced glutathione, the polyol pathway increases susceptibility to intracellular oxidative stress. The second discovery is related to intracellular production of advanced glycation end products (AGEs) precursors, which appear to damage cells by three mechanisms. The first mechanism is the modification of intracellular proteins including, most importantly, proteins involved in the regulation of gene transcription (Giardino et al., 1994; Shinohara et al., 1998). The second mechanism involves diffusion of AGE precursors out of the cell and modifies extracellular matrix molecules nearby (McLellan et al., 1994), which changes signaling between the matrix and the cell and causes cellular dysfunction (Charonis et al., 1990). The third mechanism is that these AGE precursors diffuse out of the cell and modify circulating proteins in the blood such as albumin. These modified circulating proteins can then bind to AGE receptors and activate them, thereby causing the production of inflammatory cytokines and growth factors, which intern cause vascular pathology (Li et al., 1996; Neeper et al., 1992; Smedsrod et al., 1997; Vlassara et al., 1995; Abordo, and Thornalley, 1997; Doi et al., 1992; Kirstein et al., 1992; Schimidt et al., 1995; Skolnick et al., 1991 and Vlassara et al., 1988). Animal studies show that pharmacologic inhibition of AGEs prevents late structural changes of experimental diabetic retinopathy (Hammes et al., 1991). The third mechanism was the protein
Fig. 1.3. Hyperglycemia increases flux through the polyol pathway.
kinase-C (PKC) pathway as shown schematically in Figure 1.4. In this pathway, hyperglycemia inside the cell increases the synthesis of diacylglycerol, which is critical activating cofactor for the classic isoforms of PKC -β, -δ, and -α (Koya and King, 1998; DeRubertis and Craven, 1994; Xia et al., 1994; Koya et al., 1997) (Fig. 1.4). The last mechanism was related to increased flux through the hexosamine pathway. When glucose is high inside a cell, most of that glucose is metabolized through glycolysis, going first to glucose-6-phosphate, then fructose-6-phosphate and then on through the rest of the glycolytic pathway. However, some of that fructose-6-phosphate gets diverted into a signaling pathway in which an enzyme called glutamine; fructose-6-phosphate amidotransferase (GFAT) converts the fructose-6-phosphate to glucosamine-6-phosphate and finally to uridine diphosphate (UDP)-N-acetyl glucosamine. Similar to process of phosphorylation, serine and threonine residues of transcription factors modify the N-acetyl glucosamine, and over modification by this glucosamine often result in pathologic changes in gene expression (Kolm-Litty et al., 1998; Sayeski and Kudlow, 1996; Wells and Hart, 2003) (Fig. 1.4.).

1.2 Oxidant and Antioxidant Status in Diabetes

Oxidative stress has been implicated to be an important etiological factor in the pathogenesis of diabetic complications. As discussed above, during diabetes mellitus, persistent hyperglycemia causes increased oxidative stress resulting in excessive production of free radicals (Aoki et al., 1992; Ono et al., 1998; Osterby, 1992). Diabetes mellitus therefore, is associated with increased oxidative damage of various tissues and organs due to the accumulation of lipid peroxides and AGEs (Brownlee et al., 1984; Lyons, 1992), which may lead to disruption of cellular functions and oxidative damage to membranes (Oberley, 1988). Free radicals affect the cells components such as lipid, protein, DNA and carbohydrates, of which lipids are most sensitive part. ROS can be classified in to oxygen-centered radicals and oxygen-centered nonradicals. Oxygen-centered radicals are superoxide anion (\(-\mathrm{O}_2^-\)), hydroxyl radical (\(-\mathrm{OH}\)), alkoxy radical (RO•), and peroxyl radical (ROO•). Oxygen-centered nonradicals are hydrogen peroxide (\(\mathrm{H}_2\mathrm{O}_2\)) and singlet oxygen (\(\uparrow\mathrm{O}_2\)). Other reactive species are nitrogen species such as nitric oxide (NO•), nitric dioxide (NO\(_2\)•),
Fig. 1.4. Diabetes, microvascular complications, and cardiovascular complications: what is it about glucose?
and peroxynitrite (OONO⁻) (Halliwell et al., 1995; Simon et al., 2000). ROS in biological systems are related to free radicals, even though there are nonradical compounds in ROS such as singlet oxygen and hydrogen peroxide. A free radical exists with one or more unpaired electron in atomic or molecular orbital. Free radicals are generally unstable, highly reactive and energized molecules. ROS or free radicals in biological systems can be formed by prooxidative enzyme systems, lipid oxidation, irradiation, inflammation, smoking, air pollutants, and glycoxidation (Halliwell, 1997; Stief, 2003). Several clinical studies reported that ROS are associated with many age-related degenerative diseases, including atherosclerosis, vasopasms, cancers, trauma, stroke, asthma, hyperoxia, arthritis, heart attack, age pigments, dermatitis, cataractogenesis, retinal damage, hepatitis, liver injury, and periodontis (Cohen et al., 2000; Packer and Webber, 2001). ROS also have been known to induce apoptosis of cells (Simon et al., 2000). Benign functions of free radicals have been reported, including the activation of nuclear transcription factors, gene expression and a defense mechanism to target tumor cells and microbial infections (Simon et al., 2000). Superoxide anion may serve as a cell growth regulator (Halliwell, 1997). Singlet oxygen can attack various pathogens and induced physiological inflammatory response (Stief, 2003). Nitric oxide is one of the most widespread signaling molecules and participates in every cellular and organ function in the body. Nitric oxide acts as neurotransmitter and an important mediator of the immune response (Fang et al., 2002).

Superoxide anion is a reduce form of molecular oxygen created by receiving one electron. Superoxide anion is an initial free radical formed from mitochondrial electron transport systems (Fig. 1.4). The superoxide anion plays an important role in the formation of other ROS such as hydrogen peroxide, hydroxyl radical or singlet oxygen (2·O₂⁻ + 2H⁺ → H₂O₂ + O₂) in living systems (Stief, 2003). The superoxide anion can react with nitric oxide (NO⁻) and form peroxynitrite (ONOO⁻), which can generate toxic compounds such as hydroxyl radical and nitric dioxide (ONOO⁻ + H⁺ · OH + ·NO₂) (Halliwell, 1997). Hydroxyl radical is the most reactive free radical and can be formed from superoxide anion and hydrogen peroxide in the presence of metal ions such as copper or iron (·O₂⁻ + H₂O₂ → ·OH + OH⁻ + O₂). Hydroxyl radicals have the highest 1-electron reduction potential (2310 mV) and can react with everything in
living organisms at the second-order rate constants of $10^9$-$10^{10}$/M/S (Korycka-Dahl and Richardson, 1978). In general, aromatic compounds or compounds with carbon-carbon multiple bonds undergo addition reactions with hydroxyl radicals, resulting in the hydroxylated free radicals. In saturated compounds, a hydroxyl radical abstracts a hydrogen atom from the weakest C-H bond to yield a free radical (Korycka-Dahl and Richardson, 1978). The resulting radicals can react with oxygen and generate other free radicals. Hydroxyl radicals react with lipids, polypeptides, proteins, and DNA, especially thymine and guanosine (Ashok and Ali, 1999). Hydroxyl radicals also add readily to double bonds. The barrier to the addition of hydroxyl radicals to double bonds is less than that of hydrogen abstraction, so that in competition addition is often favored. When a hydroxyl radical reacts with aromatic compounds, it can add on across a double bond, resulting in hydroxycyclohexadienyl radical (Padmaja and Madison, 1999). The resulting radical can undergo further reactions, such as reaction with oxygen, to give peroxyl radical, or decomposed to phenoxyln-type radicals by water elimination.

Hydrogen peroxide can be generated through a dismutation reaction from superoxide anion by superoxide dismutase. Enzymes such as amino acid oxidase and xanthine oxidase also produce hydrogen peroxide from superoxide anion. Hydrogen peroxide is highly diffusible and crosses the plasma membrane easily. Hydrogen peroxide is the least reactive molecule among ROS and is stable under physiological pH and temperature in the absence of metal ions. Hydrogen peroxide is a weak oxidizing and reducing agent and is thus regarded as being poorly reactive. Hydrogen peroxide can generate the hydroxyl radical in the presence of metal ions and superoxide anion ($\cdot$O$_2^-$ + H$_2$O$_2$ $\rightarrow$ $\cdot$OH + OH$^-$ + O$_2$) (Halliwell, 1997). Hydrogen peroxide can produce singlet oxygen through reaction with superoxide anion or with HOCI or chloroamines in living systems (Steif, 2000; Steif, 2003). Hydrogen peroxide can degrade certain heme proteins, such as hemoglobin, to release iron ions. Singlet oxygen is a non-radical and excited status. Takayama et al. (2001) reported that metastable phosphatidylcholine hydroperoxides present in the living organism produced singlet oxygen during their breakdown in the presence of Cu$^{2+}$ in the dark. Singlet oxygen can be formed from hydrogen peroxide, which reacts with superoxide anion or with HOCI or chloroamines in cells and tissues (Steif, 2003). Compared with
other ROS, singlet oxygen is rather mild and nontoxic for mammalian tissue (Steif, 2003). However, singlet oxygen has been known to be involved in cholesterol oxidation (Girotti and Korytowski, 2000). Oxidation and degradation of cholesterol by singlet oxygen was observed to be accelerated by the co-presence of fatty acid methyl ester. In the human organism, singlet oxygen is both a signal and a weapon, with therapeutic potency against various pathogens such as microbes, viruses, and cancer cells (Steif, 2003). Peroxyl radicals (ROO⁻) are formed by a direct reaction of oxygen with alkyl radicals (R•), for example, the reaction between lipid radicals and oxygen. Decomposition of alkyl peroxide (ROOH) also results in peroxyl (ROO⁻) and alkoxyl (RO⁻) radicals. Irradiation of UV light or the presence of transition metal ions can cause hemolysis of peroxides to produce peroxyl and alkoxyl radicals (ROOH → ROO⁻ + H⁺; ROOH + Fe³⁺ → ROO⁻ + Fe²⁺ + H⁺). Peroxyl and alkoxyl radicals are good oxidizing agents, having more than 1000 mV of standard reduction potential (Decker, 1998). They can abstract hydrogen from other molecules with lower standard reduction potential. This reaction is frequently observed in the propagation stage of lipid peroxidation. Very often the alkyl radical formed from this reaction can react with oxygen to form another peroxyl radical, resulting in chain reaction. Some peroxyl radicals break down to liberate superoxide anions or can react with each other to generate singlet oxygen (Halliwell and Gutteridge, 1985). Aromatic alkoxyl and peroxy radicals are less reactive than respective open chain radicals because of the delocalization of the electrons in the ring. Nitric oxide (NO⁻) is a free radical with a single unpaired electron. Nitric oxide is formed from L-arginine by NO synthase (Fang et al., 2002). Nitric oxide itself is not a very reactive free radical, but the overproduction of NO is involved in ischemia reperfusion, and neurodegenerative and chronic inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease. Nitric oxide, exposed in human blood plasma, can deplete the concentration of ascorbic acid and uric acid and initiate lipid peroxidation (Halliwell, 1996a). Nitric dioxide (NO₂⁻) is formed from the reaction of peroxyl radical and NO, polluted air and smoking (Nogouchi and Niki, 1999). Nitric dioxide adds to double bonds and abstract labile hydrogen atoms initiating lipid peroxidation and production of free radicals. It also oxidizes ascorbic acid (Papas, 1999). Reaction of NO and superoxide anion can generate peroxynitrite (O₂⁻ + NO⁻ → OONO⁻). Peroxynitrite is a cytotoxic species and causes tissue injury and oxidizes low density lipoprotein (Halliwell,
Peroxynitrite appears to be an important tissue-damaging species generated at the sites of inflammation (Papas, 1999) and has been shown to be involved in various neurodegenerative disorders and several kidney diseases (Knight, 1999). Peroxynitrite can cause direct protein oxidation and DNA base oxidation and modification acting as a “hydroxyl radical-like” oxidant (McVean et al., 1999). The significance of peroxynitrite as a biological oxidant comes from its high diffusibility across cell membranes (Knight, 1999). Nitrotyrosine, which can be formed from peroxynitrite-mediated reactions with amino acids, has been found in age-associated tissues (Knight, 1999).

Prooxidative enzymes, including NADPH-oxidase (Babior, 1999), NO-synthase (Stuehr et al., 1990) or the cytochrome P-450 chain (Stief, 2000), can generate ROS. Lipoxygenase generates free radicals. Lipoxygenase needs free polyunsaturated fatty acid (PUFA), which are not present in healthy tissue. Membrane-bound phospholipase produces PUFA and lysolecithins. Lysolecithins change the cell membrane structures, and free PUFA are oxidized to form lipid hydroperoxides. Lipoxygenase with Fe$^{2+}$ is inactivated status. Once Fe$^{2+}$ oxidized to Fe$^{3+}$, lipoxygenase can convert PUFA in to hydroperoxides (Spiteller, 2001). There are three major mammalian lipoxygenases: 5-, 12-, and 15-lipoxygenases (Hari et al., 2000). These enzymes can oxidize arachidonic acid, a PUFA rich in the central nervous system, in to hydroperoxyeicosatetraenoic acid. The primary localizations of 5-, 12-, and 15- lipoxygenases are in leukocytes and lymphocytes, platelets and airway cells, respectively (Hari et al., 2000). 15-lipoxygenase has been identified with in atherosclerotic lesions, which suggests that this enzyme may be involved in the in vivo formation of oxidized lipids (Knight, 1999). A serious imbalance between ROS and antioxidants causes oxidative stress. Oxidative stress is caused by antioxidant deficient diets or by increased production of ROS by environmental toxins such as those caused by smoking or by inappropriate activation of phagocytes such as with chronic inflammatory disease (Halliwell et al., 1995). Clinical studies reported that ROS are associated with many degenerative diseases, which are associated with aging (Packer and Webber, 2001). Lipid oxidation is a free radical chain reaction, and ROS can accelerate lipid oxidation (Boff and Min, 2002). Cell membranes are phospholipid bilayers with extrinsic proteins and are the direct targets of lipid oxidation (Girotti,
As lipid oxidation of cell membranes increases, the polarity of lipid-phase surface charge and formation of protein oligomers increase; and molecular mobility of lipids, number of SH groups, and resistance to thermodenaturation decrease. Malonaldehyde, one of the lipid oxidation products, can react with the free amino group of protein, phospholipids and nucleic acids leading to structural modification, which induce dysfunction of immune systems. A high level of lipid oxidation products can be detected in cell degradation after cell injury or disease. The increased lipid oxidation products are found in diabetes, atherosclerosis, liver disease, apoplexy, and inflammation. LDLs are complicated structures, and oxidative modification of LDLs has been reported to be involved with the development of atherosclerosis and CVD (Frei, 1995). Oxidized cholesterol or fatty acid moieties in the plasmatic LDL can develop atherosclerosis (Rikans and Hornbrook, 1997; Girotti, 1998; Nedeljkovic et al., 2003).

Xanthine oxidase generates $O_2^{-}$ by catalyzing hypoxanthine and xanthine to uric acid. Under pathophysiologic conditions, this is another major source of vascular oxidative stress (Droge, 2002). Xanthine oxidase exists in plasma, liver, kidney and endothelial cells but not in smooth muscle cells (Harrison et al., 2003). There is overwhelming evidence that serum xanthine oxidase levels are significantly increased in various pathological states, like hepatitis, inflammation, hypercholesterolemia, atherosclerosis, ischemia-reperfusion, carcinogenesis and aging and that free radicals generated in the enzymatic process are involved in oxidative damage (Borges et al., 2002). In hypercholesterolemic rabbits, atherosclerosis resulting from diet was ascribed to xanthine oxidase activation-induced oxidative stress (Ross, 1999). In hypercholesterolemic patients, vasodilation is improved by using the xanthine oxidase inhibitor oxypurinol. The role of xanthine oxidase in atherosclerosis is further corroborated by the following observations (Spiekermann et al., 2003): in the coronary arteries of patients with CAD, electron spin resonance studies show significant activation of both NAD(P)H oxidase and xanthine oxidase. In these patients, endothelial xanthine oxidase is inversely proportional and positively related to the effect of vitamin C on endothelium-dependent vasodilation. In asymptomatic young individuals with familial hypercholesterolemia, the increase of vascular xanthine oxidase activity is an early event. Based on the above information, it may be
possible that the inhibition of this enzymatic pathway by compounds that have both antiradical as well as xanthine oxidase inhibitory properties could provide additional therapeutic benefit. It has been reported that some flavonoids and structurally related antioxidants inhibit xanthine oxidase activity (Nagao et al., 1999; Khan and Sultana, 2004).

Aerobic metabolism is always accompanied by the production of ROS. Therefore, all aerobic organisms possess some sort of antioxidant defense with enzymatic and nonenzymatic constituents (Sies, 1993). The quantity and quality of the reactive species is determined by metabolic pathways within the organism, influenced by exogenous factors such as stress, radiation, food etc. The adverse effects of free radicals are recognized in several disorders (Feher et al., 1987), but care should be taken when assessing their causative role (Halliwell, 1994). Damage caused by free radicals is possibly involved in β-cell destruction and in the pathogenesis of diabetes mellitus (Oberley, 1988). Alterations of metabolic processes in diabetes also influence enzymatic defenses, and these changes may be associated with late complications of diabetes. Antioxidant enzymes primarily account for intracellular defense, while several nonenzyme molecules, small molecular weight antioxidants, protect various components against oxidation in plasma. Antioxidant enzymes including superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase, convert ROS in to non-reactive oxygen molecules. Intracellular antioxidant defense is primarily provided by antioxidant enzymes, which catalyze decomposition of ROS. The three major antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (Gpx) and catalase, differ from each other in structure, tissue distribution and cofactor requirement. SOD catalyzes the conversion of superoxide anion to hydrogen peroxide and oxygen. SOD activity was discovered by McCord and Fridovich in 1969; they later proved that the enzyme is required to sustain life in aerobic conditions (McCord et al., 1971). Several classes of the enzyme have since been specified, each containing a transition metal in its catalytic centre. In humans, mitochondrial MnSOD, and extra-and intracellular-CuZnSOD have been identified. Gpx is a selenium-dependent enzyme (selenoprotein). The extracellular form is a glycoprotein; the intracellular and mitochondrial forms also possess different antigenic structures. The substrate of the enzyme is reduced glutathione.
(GSH), and therefore it depends indirectly on the flavoprotein glutathione reductase (Gred) and cellular NADPH concentration. Gpx uses a specific H donor, GSH, for the reduction of non-specific substrates (hydrogen peroxide, lipid and non-lipid hydroperoxides). The enzyme contains seleno-cysteine in its active centre, which is incorporated into the polypeptide chain during translation. Selenium deficiency, both in vitro and in vivo, leads to enzyme deficiency. Thus, when assessing GPX function, it may be necessary to examine selenium status and free GSH concentration, at least when looking for the cause of altered activity. Catalase is a heme-containing ubiquiter enzyme, in eukaryotes it is found in peroxysomes. The enzyme probably serves to degrade hydrogen peroxide produced by peroxisomal oxidases to water and oxygen. Several other enzymes are also involved in the prevention of oxidative damage or its repair, such as Gred, enzymes of NADPH production and DNA repair enzymes. Different cell types and cellular compartments contain antioxidant enzymes in varying quantities. Regulation of antioxidant enzyme activity in eukaryote organisms may be influenced by such factors as age, hormonal state, organ specificity, and amount of cofactors present (Harris, 1992).

The glutathione-S-transferases (GSTs) are a family of multifunctional proteins that function both as important enzymes of detoxification and intracellular binding proteins (Boyer, 1989). As enzymes, they catalyze the reaction between nucleophil reduced GSH and large number of electrophilic compounds such as polycyclic aromatic hydrocarbons, aromatic amines, azodyes, alkylation agents, carcinogens and neurotoxins (Boyland and Chasseaud, 1969; Habig et al., 1974; Jackoby, 1978; Chasseaud, 1979). They also bind a number of amphipathic compounds that they do not metabolize (non-substrate ligands) and have been suggested to act as intracellular transport proteins for compounds that have limited solubility in water (Levi et al., 1969). Additionally, a number of endogenous compounds, including prostaglandins, leucotrienes, organic hydroperoxides (including lipid hydroperoxides and products of lipid peroxidation) and steroids act as substrate for GST (Mannervik, 1985; Jackoby, 1978; Chasseaud, 1979; Kaplowitz, 1980). However, it is important to note that hydrogen peroxide is not a substrate for GST (Lawrence and Burk, 1976). Two types of products are produced by GST catalyzed reactions (Douglas, 1987). In one type of reaction, a stable glutathione conjugate is formed by the nucleophilic attack of GSH
on an electrophilic centre. These types of reactions occur with substrates such as epoxides (metabolites of benzo (a) pyrine and aflatoxin A) alkyl and amyl halides (sulfobromophthalein) and reactions with reactive products of P450-catlayzed reactions (acetaminophen), to name just a few. In the second type of reaction, a reduced substrate and glutathione disulfide (GSSG) are formed. In this second type of reaction an unstable intermediate is the enzymatic product, which is attached nonenzymatically by a second molecule of GSH, yielding the final product and GSSG. Examples of substrates for this second type of reaction are organic nitrates and organic hydroperoxides. The resulting glutathione conjugates that formed are more soluble than the original substrates and thus more easily exported from the cell. The release of glutathione-S-conjugates from cells is an ATP-dependent process mediated by membrane glycoproteins belonging to multidrug-resistance protein (MRP) family. Proteins of the MRP family are essential for the transport of glutathione-S-conjugates into the extracellular space. They are also known as glutathione-S-conjugate pumps (Hayes and Strange, 2000).

In animals including humans, and in plants glutathione (GSH) is the predominant non-protein thiol and functions as a redox buffer, keeping with its own SH groups those of proteins in a reduced condition among other antioxidant activities. GSH acts as a reducing agent and a vital substance in detoxification, also provides antioxidant protection in the aqueous phase of cellular systems (Rana et al., 2002); its antioxidant activity is through the thiol group of its cysteine residue. Like ascorbic acid another important water soluble antioxidant, GSH directly reduce a number of ROS and is oxidized to GSSG in this process. The liver is the principal site of GSH synthesis. In healthy tissue, more than 90 % of the total glutathione pool is in the reduced form and less than 10 % exists in the disulfide form. The enzyme glutathione disulfide reductase is the principal enzyme that maintains glutathione in its reducing form. This latter enzyme uses as its cofactor NADPH, which is generated by the oxidative reaction in the pentose phosphate pathway. GSH/GSSG ratio is the indicator of redox status of the tissues (Meister, 1992; Meister and Anderson, 1983; Schafer and Buettner, 2001). Intrahepatic glutathione is reported to afford protection against liver dysfunction by at least two ways: (i) as a substrate of Gpx, GSH serves to reduce large variety of hydroperoxides before they attack unsaturated lipids or convert
already formed lipid hydroperoxides to the corresponding hydroxyl compounds; (ii) as a substrate of GST, it enables the liver to detoxify foreign compounds or their metabolites and to excrete the products, preferably into the bile. The consequences of a functional glutathione deficiency, which results in tissue oxidative stress, can be seen in some pathological conditions. For example, those with glucose-6-phosphate dehydrogenase deficiency produce lower amounts of NADPH and hence, lower amounts of GSH. This condition is characterized by hemolytic anemia. Conditions causing chronic glutathione deficiency all result in hemolytic anemia, among other pathological consequences. Oxidative stress caused by glutathione deficiency results fragile erythrocyte membranes. Chronic functional glutathione deficiency is also associated with immune disorders, an increased incidence of malignancies and in the case of HIV disease probably accelerated pathogenesis of the disease (Hercbergs et al., 1992; Holoroyd et al., 1993). Glutathione has also been shown to enhance insulin secretion in elderly subjects with impaired glucose tolerance (Paolisso et al., 1992).

1.2.1 Antioxidant enzyme activity alterations in diabetes mellitus

The metabolic alterations that accompany diabetes mellitus can affect prooxidant-antioxidant balance in several ways.

(i) Induction: Response to Oxidative Stress: There are several proposed mechanisms of free radical production in diabetes, glycoxidation (Baynes, 1991; Wolff and Dean, 1987), change in intracellular NADH/NAD ratio (hyperglycemic pseudohypoxia) (Williamson et al., 1993), and effects on prostaglandin biosynthesis (Tesfamariam, 1994). Since expression of antioxidant enzymes may be induced at the transcriptional level by oxidative stress (Kullik and Storz, 1994), it is possible that the metabolic changes accompanying diabetes may induce these enzymes. (ii) Allosteric Effects: Influence of Glycation on Enzyme Activity: The characteristic feature of diabetes, hyperglycemia, enhances nonenzymatic binding of glucose to proteins: the glycation phenomenon causes structural and functional changes in the proteins like hemoglobin, albumin, lens crystalline proteins, basal membranes of glomeruli, etc. Thus, high extra-and intra-cellular concentration of glucose could cause glycation and consequently functional changes of the antioxidant enzymes. (iii) Activation-inactivation: Changes in the Concentration of Cofactors: SOD requires manganese or
copper and zinc, Gpx needs selenium, and catalase contains heme as cofactor. Diabetes may influence antioxidant enzyme activity through disturbances in micronutrient status (Strain, 1991). The distribution and function of ions of vital importance may change in diabetes, and the potassium and calcium channels may work differently. Changes in iron metabolism leads to changes at the cofactor level. In a long-term experiment, Wohaieb and Godin (1987) observed a decrease of SOD activity in the liver and kidney and an increase in the pancreas of STZ-treated diabetic rats. They proposed that the increase in enzyme activity might be an adaptive response in the otherwise SOD-poor pancreas, while the reduction of SOD activity in liver and kidney might be due to the direct damaging effect of free radicals on the enzyme. Dohi et al. (1988) found no difference in the SOD activity of the kidneys in STZ-treated diabetic rats after four months of diabetes. Matkovics et al. (1982) observed decreased SOD activities in liver, kidney, spleen, brain, heart, muscles and pancreas, except lungs of STZ-and alloxan-treated diabetic rats. Loven et al. (1986) observed a decrease in CuZnSOD activity in liver, kidney and erythrocytes after 10 days of STZ-induced diabetes. Sukalski et al. (1993) described the decrease of liver mitochondrial SOD activity in diabetic rats. A significant decrease of CuZnSOD activity in diabetic rabbit aorta endothelium was reported by Tagami et al. (1992), although others have not found any difference in aortic SOD activity between diabetic and control rats (Langenstroer and Pieper, 1992). Red blood cell SOD is frequently measured in humans as an index of defense against superoxide in blood. In diabetics, activity of erythrocytes SOD has been shown to be decreased (Matkovics et al., 1982) increased (Godin et al., 1988) and unchanged (Bono et al., 1987; Kaji et al., 1985). Kawamura et al. (1992) showed that red blood cell CuZnSOD is glycated both in vitro and in vivo, leading to its inactivation, and the percentage of this glycated SOD is higher in type 1 diabetic children than in healthy individual. The percentage of extracellular glycated SOD has also been found to be higher in diabetics (Adachi et al., 1991), but its activity was comparable to that of the unmodified enzyme. Glycation was shown to affect the C-terminal end of the enzyme reducing its heparin-binding affinity. Thus protection against extracellular radicals by cell surface attached SOD may be impaired in diabetes, leaving the endothelium more susceptible to damage by superoxide anion.
Mukherjee et al. (1994) observed a significant reduction of GSH content after 15 days, and reduction of Gred activity after 3-weeks in liver, kidney, brain and blood of STZ-treated diabetic rats. Others have reported reductions in mitochondrial Gpx and Gred activity in liver of diabetic rats (Sukalski et al., 1993). Loven et al. (1986) studied intestinal mucosa and liver GSH content after 10 days of STZ-induced diabetes; there was a significant decrease of GSH in the liver while no change was noticed in the mucosa. Orally administered GSH restored, and intramuscular insulin even increased liver GSH above normal levels. Abnormal GSH synthesis was thought to be responsible for the changes. Wohaieb and Godin (1987) found that in the liver, which normally contains high amount of GSH and strong Gpx activity, induction of diabetes caused a decreased in both the parameters. While in the kidney, which is relatively poor in Gpx activity, diabetes led to an increase in activity. Insulin treatment reversed these alterations. Gred activity was shown to be increased in erythrocytes of spontaneously diabetic BB rats, while in alloxan-treated animals Gpx activity was also increased (Godin et al., 1988). These workers also found similarly elevated erythrocytes Gred levels and resistance to peroxide-induced GSH reduction in type 1 and type 2 diabetic patients. They proposed that elevated glucose levels could increase NADPH production resulting in a more effective GSH reduction (Godin et al., 1988). Dohi et al. (1988) noticed significant reduction in Gpx activity in aorta homogenates of rats, 4 and 8 months after induction of diabetes. They also found higher serum selenium concentrations in diabetic rats. Tagami et al. (1992) found reduced GSH content and Gpx activity and unchanged Gred activity in aortic endothelial cells of diabetic rabbits. Langenstroer and Pieper (1992) found no alteration in Gpx activity in diabetic rat aorta. Blakynny and Harding (1992) observed that incubation of cow erythrocytes with glucose, glucose-6-phosphate and fructose results in a time-dependent reduction of Gred activity. The experiment suggested that glycation of the enzyme was responsible for the observed decrease. Decreased Gred activity in erythrocytes of diabetic children was reported by Stahlberg and Hietanen (1991), but Walter et al. (1991) found no difference between Gpx and Gred activity of diabetics and nondiabetics. Murakami (1991) examined erythrocytes of diabetics (fasting glucose level > 140 mg/dl) and concluded that GSH reduction and glutathione increase in erythrocytes was caused by defective functioning of γ-glutamyl-cysteine synthase due to its glycation, decrease in Gred activity and defect in glutathione
transport. Yoshida et al. (1995) confirmed that in erythrocytes of poorly controlled diabetics (HbA1 = 10.6 ± 1.3 %) GSH synthesis and thiol transport is impaired, and cells become susceptible to oxidative damage. Jain and McVie (1994) found that erythrocytes GSH content was negatively correlated to HbA1, a good estimate of long-term hyperglycemia in diabetics. In diabetic patients with explicit hyperglycemia (HbA1 = 11.5 ± 1.9 %), Uzel et al. (1987) found impaired Gpx activity and lower erythrocytes GSH, in addition, to elevated lipid peroxidation products, the alterations being more pronounced in patients with retinopathy. Reduced GSH and protein-SH content in erythrocytes of diabetics was reported by Bono et al. (1987). Kaji et al. (1985) reported no difference in erythrocytes Gpx activity but an increase in plasma Gpx activity of diabetic compared to nondiabetic women. Elevation of serum selenium levels and Gpx activity in diabetic children was reported by Cser et al. (1993). Similarly, higher Gpx activity has been reported in erythrocytes of diabetics (Matkovics et al., 1982).

Observations regarding catalase activity in the vasculature are rather controversial. Tagami et al. (1992) reported a decrease in aortic endothelial cell catalase activity in diabetic rabbits, which was restored by insulin. Dohi et al. (1988) observed no alteration in catalase activity of rat aorta homogenate, while Langenstroer and Pieper (1992) reported its increase. The activity of catalase in liver and kidneys of diabetic animals is generally believed to decrease (Wohaieb and Godin, 1987; Godin et al., 1988; Asayama et al., 1989), although there are also reports of its increase (Dohi et al., 1988; Matkovics et al., 1982). On the other hand, heart and pancreas tissue show increased catalase activity in the diabetic state (Wohaieb and Godin, 1987; Godin et al., 1988; Asayama et al., 1989). Erythrocytes catalase activity seems not to be altered either in diabetic animals or in type 1 and type 2 diabetic patients (Wohaieb and Godin, 1987; Matkovics et al., 1982; Godin et al., 1988; Bono et al., 1987; Kaji et al., 1985). It is apparent from the above reports that contradictory changes in the activities of particular enzymes in particular organs have been observed in many cases. These discrepancies may be partly explained by the variability in the diabetes models used, including the strain and sex of the animals, their age at the induction of diabetes, the severity of the resulting insulin deficiency, and the duration of diabetes. For the clinical observations similar confounding factors
exist, such as the type and duration of diabetes, mode of treatment, presence or absence of complications, which are not revealed by routine laboratory tests. Changes in enzyme activity (increased, impaired, unchanged) may depend on the above mentioned factors to a large extent. In type 1 diabetes, when blood glucose is strictly controlled by intensive insulin therapy, is not accompanied by remarkable changes in the prooxidant-antioxidant balance. It has been reported that erythrocytes SOD and catalase as well as whole blood Gpx activities of patients with well controlled type 1 diabetes do not differ from those of healthy controls (Szaleczky et al., 1997). In an investigation of relationship between the duration of diabetes and various measures of antioxidant activity in blood, it was found that erythrocytes SOD was reduced in patients who had had diabetes for more than 10 years compared to patients whose disease was not so long-standing, while whole blood Gpx and erythrocytes catalase activities did not differ (Prechl et al., 1997). In recent years it has been definitely shown that lesser the diabetic metabolic control, higher the frequency of late complications (DCCTRG, 1993). Should oxidative stress play a role in the development of these complications, one would expect adaptive changes to be observed in the antioxidant defense system?

Several abnormalities, such as reduced life span (Jones and Peterson, 1981), increased viscosity (Satoh et al., 1984), excessive aggregation (Schmid-Schonbein and Volger, 1976), and increased tendency to adhere to endothelial cells (Wali et al., 1988) have been reported in the erythrocytes of diabetic patients. Changes in the levels of phospholipids (Faas and Carter, 1983), cholesterol (Dang et al., 1984), cholesterol to phospholipid molar ratio (Miossec et al., 1999), unsaturated fatty acids (Ruiz-Gutierrez et al., 1993) and altered membrane phospholipid asymmetry (Wali et al., 1988) have also been reported in the erythrocyte membranes of diabetic patients. Alterations in lipid composition can affect the physico-chemical properties of the erythrocytes membrane (Shiga et al., 1979), including plasma membrane Na⁺, K⁺-ATPase and Mg⁺⁺-ATPase activities (Tsakiris and Deliconstantinos, 1984). A decrease in the plasma membrane Na⁺, K⁺-ATPase activity has been reported in erythrocyte membranes of diabetics (Mimura et al., 1994). A defect in the ouabain-sensitive Na⁺, K⁺-ATPase of erythrocytes from STZ-diabetic rats has also been reported. This defect was accompanied by an increase in cell volume an osmotic
fragility and a decrease in the cytosolic K\(^+\)/Na\(^+\) ratio. As a consequence of which Na\(^+\), K\(^+\)-ATPase activity in erythrocytes membrane fragments from diabetic rats was significantly reduced. On the other hand, Mg\(^{++}\)-ATPase activity was not diminished in erythrocytes of diabetic rats (Kowluru et al, 1989). Insulin, within a physiological range of concentration normalizes lipid composition and order in erythrocytes in diabetic patients (Kowluru et al, 1989). It stimulates Na\(^+\), K\(^+\)-ATPase activity and translocation to plasma membrane via phosphorylation of the \(\alpha\)-subunits by protein kinases (Kowluru et al, 1989; Al-Khalili et al., 2004). Erythrocytes are exposed to continuous oxidative stress, because oxygen radicals are continuously generated by the auto-oxidation of hemoglobin (Misra and Fridovich, 1972). Oxygen radicals formed over and above the detoxifying capacity of erythrocytes can cause peroxidative breakdown of phospholipids, fatty acids and accumulation of malondialdehyde (Halliwell and Gutteridge, 1984). Erythrocytes of diabetic patients and smokers are more susceptible to lipid peroxidation, when treated with hydrogen peroxide \textit{in vitro}. (Uzel et al., 1987; Ismail et al., 2002).

1.3 Cholesterol Dynamics in Diabetes/Hyperlipidemia

Cholesterol, which is widely distributed in the animal kingdom, occurs in free form (unesterified) in all cell membranes (Myant, 1981) while in the plasma most of the cholesterol occurs in esterified form. Cholesterol has many biological functions. For instance, the concentration of cholesterol influences the fluidity of cell membranes and thereby biological activities of the cell. The cholesterol acts as a precursor for the synthesis of bile acids and steroid hormones. The total body cholesterol is derived from two sources: (i) dietary and (ii) \textit{de novo} biosynthesis. Cholesterogenesis mostly occurs in the liver, which also regulates the level of circulating plasma cholesterol and serum lipoproteins. The biosynthesis of cholesterol also occurs in the other organs like the intestines, adrenal cortex, reproductive organs and skin. Although other cells and tissues do not synthesize cholesterol, they have the genomic information for its synthesis. Under normal circumstances, these cells and tissues take up cholesterol from serum lipoproteins.

Starting from the 2-carbon unit acetyl-CoA, the biosynthesis of the cholesterol proceeds to several intermediates, including mevalonate and isopentenyl
pyrophosphate (Fig. 1.5). Isopentenyl pyrophosphate is further processed in a series of steps to two branched pathways, one leading to isopentenyl tRNA and isopentenyl adenine, and the other to farnesyl pyrophosphate. Farnesyl pyrophosphate is in turn channeled to synthesis of cholesterol, ubiquinone or dolichols (Ross and Glomset, 1973; Brown and Goldstein, 1983). The observation that cancer cells lose feedback control of cholesterol biosynthesis, show elevated cholesterol levels, and exhibit a higher rate of cholesterogenesis provided the first indication of the link between cholesterol biosynthesis and cancer cell growth (Coleman and Laviets, 1981). The subsequent realization that not only cancer cells but also preneoplastic and normal proliferating cells show elevated levels of cholesterol as well as higher rates of cholesterogenesis indicated that cholesterol biosynthesis is likely to play an important role not only in carcinogenesis but also in normal cell growth (Rao, 1986). Hepatic level of cholesterol are maintained by a precised balance between reactions catalyzed by 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase, EC 1.1.1.34), cholesterol 7-α-hydroxylase, acyl CoA: cholesterol acyltransferase and cholesteryl ester hydrolase. The first two enzymes are the rate limiting enzymes for cholesterol and bile acid synthesis, respectively. Acyl CoA: cholesterol acyltransferase catalyzes esterification of free cholesterol, whereas cholesteryl ester hydrolase mediates release of free cholesterol from stored cholesteryl esters.

The primary feedback loop for regulation of cholesterol synthesis appears to be at the site where HMG-CoA is converted to mevalonic acid by the rate-limiting enzyme HMG-CoA reductase, cholesterol and other oxysterols inhibit the activity of HMG-CoA reductase. Because of the strong link between cholesterol and CHD and its link with cancer (Rao, 1986), there is currently a renewed interest in studying the regulation of HMG-CoA reductase and several other key enzymes in cholesterol biosynthetic pathway. HMG-CoA reductase regulates the synthesis of cholesterol and other polyisoprenoid compounds (Rodwell et al., 1976; Goldstein and Brown, 1977; Brown and Goldstein, 1979; Brown and Goldstein, 1980; Beg et al., 1981; Beg and Brewer, 1982). In mammalian cells, HMG-CoA reductase is a transmembrane glycoprotein with its active site facing the cytosol and a carbohydrate containing site oriented toward the luminal surface of the endoplasmic reticulum (Liscum et al., 1983; Brown and Simoni, 1984). HMG-CoA reductase is an approximately 100 kDa
Fig. 1.5. The biosynthetic pathways of mevalonate, sterols and isoprenoid compounds in mammalian cells.
protein (Chin et al., 1982; Edwards et al., 1983a; Hardeman et al., 1983; Chin et al., 1984; Beg et al., 1985). Proteolysis of the native protein results in a 53 kDa molecular weight fragment that contains the active site of the enzyme (Chin et al., 1982; Liscum et al., 1983a; Edwards et al., 1983a; Hardeman et al., 1983; Chin et al., 1984; Beg et al., 1985). HMG-CoA reductase is a protein of 887 amino acids containing three potential sites for asparagine-linked glycosylation. The N-terminal half of the peptide is anchored to the membrane and contains seven hydrophobic regions, each of which is comprised of 20 amino acids and spans the microsomal membrane (Chin et al., 1984). The N-terminal lacks the signal sequence and the hydrophilic C-terminal half of HMG-CoA reductase contains the catalytic site of the enzyme (Chin et al., 1984). Since, the catalytically active hydrophilic tail of the enzyme extends into the cytoplasm, it is more accessible to the action of modulators and permits the observed multifaceted regulation of HMG-CoA reductase and cholesterol synthesis. The complex homeostatic mechanism by which the enzyme activity of HMG-CoA reductase and cholesterol biosynthesis are coordinately regulated in response to various physiological stimuli has been extensively studied. Isolation, purification, and characterization of rat hepatic HMG-CoA reductase have been well documented (Kawachy and Rudney, 1970; Heller and Gould, 1973; Brown et al., 1973; Heller and Gould, 1974; Heller and Shrewsbury, 1976; Kleinsek et al., 1977; Srikantaiah et al., 1977; Edwards et al., 1979). HMG-CoA reductase has also been studied in several other species including chicken liver (Beg et al., 1978; Beg et al., 1979), human liver (Beg et al., 1982; Beg et al., 1984) and human fibroblasts (Brown and Goldstein, 1983; Beg et al., 1987). Several different mechanisms for the regulation of enzymes in metabolic pathways have been elucidated such as modulation by isosteric and allosteric effectors, regulation of enzyme synthesis and degradation, feedback control, and covalent modification (Siperstein, 1970; Holzer and Duntze, 1971; Segal, 1973; Carlson and Kim, 1973; Lee et al., 1976; Greengard, 1978). Three basic control mechanisms for HMG-CoA reductase have been reported. (a) Long-term regulation, which involves the modulation of HMG-CoA reductase activity by changes in enzyme concentration through the regulation at transcriptional level and post-transcriptional regulatory mechanisms such as mRNA and enzyme protein degradation (Kirsten and Watson, 1974; Jakoi and Quarfordt, 1974; Chang et al.,
1981; Koizumi et al., 1982; Faust et al., 1982; Edwards et al., 1983a; Edwards et al., 1983b; Liscum et al., 1983b; Clarke et al., 1983; Sinensky and Logel, 1983; Clarke et al., 1984). For instance, the product feedback regulation by mavalonate (Kita et al., 1987; Brown and Goldstein, 1980; Cohen et al., 1982), in vivo inhibition of HMG-CoA reductase by cholesterol feeding (Arebalo et al., 1981), cholesterylamine and mevinolin (Eisenberg and Levy, 1975), and mevalonolactone (Arebalo et al., 1980; Beg et al., 1984) has been reported. Tocotrienols, a naturally occurring class of compounds of vitamin E family, have also been reported to regulate HMG-CoA reductase activity at the post-transcriptional level (Pearce et al., 1992; Parker et al., 1993). (b) Control of HMG-CoA reductase activity through changes in the membrane composition and membrane fluidity in the microsomal environment in the immediate vicinity of the enzyme (Finkel and Volpe, 1979; Mitropoulos et al., 1981; Siptal and Sabine, 1981; Richert et al., 1984). (c) Short-term regulation that involves reversible covalent modification (phosphorylation and dephosphorylation) of HMG-CoA reductase (Ingebritson and Gibson, 1980; Beg et al., 1981; Beg and Brewer, 1982; Kennelly and Rodwell, 1985). Three separate kinase systems for the regulation of HMG-CoA reductase involving short-term covalent modification have been demonstrated (Ingebritson and Gibson, 1980, Beg et al., 1987). Studies involving incubation of rat hepatocytes with insulin and glucagon or administration of glucagon to rats have been shown to modulate the bicyclic cascade system involving phosphorylation of both HMG-CoA reductase and HMG-CoA reductase kinase (Ingebritson and Gibson, 1980; Gibson, 1985; Beg et al., 1987; Gibson and Parker, 1987).

Although cholesterol is essential to life, excess or deficit of free cholesterol is known to be harmful. Several factors are known which cause an overall increase in cholesterol concentration in the liver (i) uptake of lipoproteins by receptor mediated endocytosis, (ii) non-receptor mediated intake of lipoproteins, (iii) uptake of free cholesterol from the cholesterol rich lipoproteins by cell membranes, (iv) de novo synthesis of cholesterol, and (v) hydrolysis of cholesterol esters by cholesteryl ester hydrolase. Under above situations, not only increased cholesterol levels inhibits its own synthesis by inhibiting HMG-CoA reductase and suppressing low density lipoprotein (LDL) receptors (Russel et al., 1983), but also by activating cholesterol 7-
α-hydroxylase and acyl CoA: cholesterol acyltransferase which utilize free cholesterol for bile acid synthesis and formation of cholesteryl esters, respectively. Conversely, some factors are involved in the decrease of hepatic cholesterol, they are (a) efflux of cholesterol from membrane to nascent high-density lipoproteins (HDL) and HDL₃ which is catalyzed by lecithin: cholesterol acyltransferase, (b) esterification by acyl CoA: cholesterol acyltransferase and (c) utilization of cholesterol for synthesis of steroids and bile acids. Under these conditions, increase in cholesterol is achieved by activation of HMG-CoA reductase and cholesteryl ester hydrolase activities as well as induction in synthesis of LDL receptors in order to receive cholesterol from non-hepatic tissues by receptor mediated endocytosis. However, under normal conditions an intricate balance is maintained between the biosynthesis, utilization and transport of cholesterol, keeping its harmful effects to minimum.

Lipids are transported through plasma compartment in lipoproteins, which are complex water soluble molecules consisting of a core of cholesteryl esters and TG covered by a surface monolayer of phospholipids, free cholesterol and apolipoproteins. In the last two decades, there have been major advances in our understanding of the role of plasma lipoproteins, apolipoproteins, lipolytic enzymes, and lipoprotein receptors in cholesterol and lipoprotein metabolism. This new information has provided major insights into the role of cholesterol and lipoproteins in the pathogenesis of premature atherosclerosis. There are six major classes of human plasma lipoproteins, these include chylomicrons, very low density lipoproteins (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL), high density lipoprotein (HDL) and lipoprotein (a) [Lp(a)] (Gofman et al., 1954; Berg et al., 1974). HDL can be further separated by hydrated density into HDL₂a, HDL₂b, HDL₃a, HDL₃b and HDL₃c, (Kontush et al., 2003). These lipoproteins are distinguished on the basis of their lipid content, ultracentrifugation size, electrophoretic mobility and surface proteins. Fourteen major human plasma apolipoproteins have been identified and their gene and protein structures determined (Brewer et al., 1988). The five most clinically relevant apolipoproteins are A-I, B-100, B-48, C-II and E. The two major apolipoproteins on HDL are apoAI and apoAII (Suzuki et al., 1983). In human plasma apoB exist as two isoproteins designated apoB-100 and apoB-48, with molecular weights of 512 and 250 kDa, respectively.
ApoB-48 and apoB-100 are the principal structural apolipoproteins on chylomicrons, VLDL, IDL and LDL. ApoB-100 is virtually the only apolipoprotein on LDL. Three major physiological functions for the plasma apolipoproteins have been identified. (i) Apolipoproteins function as structural proteins for the biosynthesis and secretion of plasma lipoproteins. ApoB-100 and apoB-48 are required for the secretion of TAG-rich lipoproteins from the liver and intestine. ApoA-I has been proposed to be an important structural protein for the biosynthesis of HDL. Individuals with an inability to synthesize and secrete apoA-I have a virtual absence of plasma HDL (Schaefer et al., 1985). (ii) Apolipoproteins function as cofactors or activators of enzymes involved in lipid and lipoprotein metabolism. ApoC-II is required for the enzymatic activity of lipoprotein lipase, which is responsible for the perivascular hydrolysis of lipoprotein TAG to free fatty acids and monoacylglycerols (La Rosa et al., 1970; Havel et al., 1970). Lipoprotein lipase is attached to the capillary endothelium by a heparin-like proteoglycan allowing direct interaction of the enzyme with the circulating TAG-rich lipoproteins. A deficiency of lipoprotein lipase or apoC-II results in defective TAG hydrolysis (Breckenridge et al., 1978). Clinically, patients have eruptive xanthomas, severe hypertriglyceridemia and recurrent bouts of pancreatitis. ApoA-I activates lecithin: cholesterol acyltransferase, which catalyzes the esterification of plasma cholesterol to cholesteryl esters (Fielding et al., 1972). (iii) Apolipoproteins also play a critical role in lipoprotein metabolism as ligands on lipoprotein particles, which interact with cellular receptors for specific lipoproteins. ApoB-100 interacts with the LDL receptor to initiate absorptive endocytosis and cellular uptake of LDL (Brown and Goldstein, 1986). ApoE has been proposed to interact with apoE receptor, which facilitates the hepatic removal of lipoprotein remnants, secreted by the intestine and liver (Hertz et al., 1988).

The metabolic relationship of the major classes of lipoproteins containing apoB-48 and apoB-100 may be considered to consist of two major “apoB cascades”. The first apoB cascade involves the stepwise delipidation of TG-rich chylomicrons secreted by the intestine. These lipoproteins transport dietary cholesterol and TG from the intestine to the liver and peripheral tissues. Shortly after secretion, chylomicrons acquire apolipoproteins C-II and E primarily from HDL. As already outlined, apoC-II
activates lipoprotein lipase, which initiates TG hydrolysis and remodeling of the lipoprotein particles. With TG hydrolysis of the hydrated density the chylomicrons increases and chylomicron remnants are generated with a hydrated density of VLDL and then IDL. Chylomicron remnants are removed primarily by a hepatic remnant receptor. ApoE has been proposed to interact and initiate the hepatic uptake of the chylomicron remnants (Hertz et al., 1988). The second apoB cascade is a parallel cascade involving TG rich VLDL containing apoB-100 secreted by the liver. ApoC-II and apoE from HDL rapidly associate with the newly secreted hepatogenous VLDL. ApoC-II activates lipoprotein lipase, which hydrolyzes VLDL triglycerides, and the VLDL is serially converted to smaller VLDL remnants, IDL, and finally LDL. During the conversion of VLDL to LDL approximately 50 per cent of VLDL remnants and IDL are removed directly from the plasma by interaction of apoE and apoB-100 with the remnant and LDL receptors.

Another lipolytic enzyme, hepatic lipase and apoE have also been proposed to be necessary for the conversion of IDL to LDL. Hepatic lipase functions as both a triglyceryl hydrolase and phospholipase. LDL, the end product of the VLDL cascade, contains almost exclusively apoB-100 as its only protein constituent. ApoB-100 on LDL interacts with LDL receptor on the plasma membrane of cells in the liver, adrenal and peripheral cells, including smooth muscle cells and fibroblasts (Brown and Goldstein, 1986), where it supplies cholesterol to the intracellular cholesterol pool. The work of Brown and Goldstein (1979 & 1983) on the cellular metabolism of LDL elucidated the LDL Pathway. The high affinity receptors bind LDL particles and extract them from the fluid that bathes the cell. LDL is transported to lysosomes where the protein is degraded and the cholesterol is transferred to the intracellular cholesterol pool (Brown and Goldstein, 1986). The receptor displayed on the surface of the cells varies with the cellular demand for cholesterol. When the need is low, the cells make fewer receptors and take up LDL at a reduced rate, this protects the cells excess cholesterol but at a higher price, the reduction in the number of receptors decreases the rate of removal of LDL from the circulation, blood level of LDL rises and atherogenesis is accelerated. LDL receptor plays an important role in the maintenance of plasma LDL-cholesterol levels. The serum concentration of LDL, therefore, depends on the rate that liver removes IDL from the circulation, which in
turn, depends on the number of functioning LDL receptors on the liver cell surface. High blood cholesterol which results from the overproduction and/or underutilization of LDL, is known to be caused by two metabolic irregularities: (i) the genetic disease familial hypercholesterolemia (discussed in detail later); (ii) the consumption of high cholesterol diet. Familial hypercholesterolemia (FH) is a dominant genetic defect that results in a deficiency of functional LDL receptors. FH homozygotes, therefore have plasma LDL-cholesterol levels three to five times higher than average. FH heterozygotes, which are far more common, have about one half of the normal number of functional receptors and plasma LDL-cholesterol levels of about twice the average. The ingestion of high cholesterol has an effect similar, although not as extreme, as FH. Excessive dietary cholesterol enters the liver cells in chylomicron remnants and represses the synthesis of LDL-receptor protein. The resulting insufficiency of LDL-receptors on the liver cell surface has consequences similar to those of FH. In Watanable hereditable hyperlipidemic (WHHL) rabbits, with a genetic deficiency of LDL receptor function, extremely high plasma LDL cholesterol levels are observed with the development of atherosclerosis early in life (Brown and Goldstein, 1983). LDL receptor activity is under the metabolic regulation in vivo, such that receptor activity can be increased or decreased by appropriate interventions with diet and/or drugs (Mahley and Innerarity, 1983; Brown and Goldstein, 1983).

Nascent HDL, primarily in the form of phospholipid-apolipoprotein A-I discs, are synthesized both in the human liver and intestine. As cell of the body die and as cell membranes undergo turnover, free cholesterol is continually released in to the plasma. This cholesterol is immediately adsorbed on to high-density lipoproteins (HDL: diameter, 5 to 12nm), and in this location it is esterified with a long-chain fatty acid by an enzyme in plasma, lecithin: cholesterol acyltransferase (LCAT). The newly formed cholesteryl esters are rapidly transferred from HDL to VLDL or IDL particles by a cholesteryl ester transfer protein in plasma. The IDL particles are ultimately taken up by the liver or converted to LDL. The nascent HDL acquires cholesterol from tissues and the enzyme lecithin: cholesterol acyltransferase catalyzes the esterification of cholesterol to cholesteryl esters. With the increase in lipid content, the nascent HDL are converted to HDL₃, these HDL₃ lipoproteins are then converted to the larger HDL₂ lipoproteins by the acquisition of lipids and apolipoproteins
released during the stepwise delipidation and remodeling of the TG rich chylomicrons and VLDL as well as the uptake of cholesterol from peripheral tissues. HDL₂ is converted back to HDL₃ by the removal of TAG and phospholipids by hepatic lipase as well as by the transfer of cholesteryl esters into VLDL and LDL by the cholesteryl ester transfer protein (CETP) as well as the transfer of cholesteryl esters to the liver and other tissues. In this overall process, HDL are interconverted from HDL₃ to HDL₂ and back to HDL₃ as cholesterol is picked up and transferred from peripheral tissues to the liver. This process is termed as reverse cholesterol transport (Brewer et al., 1971; Eisenberg et al., 1984). In this proposed model, HDL interacts with a putative HDL receptor (Suzuki et al., 1983; Schmitz et al., 1988) that facilitates the transfer of intracellular cholesterol to HDL. HDL transports this cholesterol in plasma and delivers it to the liver via the HDL receptor for removal from the body by direct secretion into bile or following conversion to bile acids. A variable portion of tissue cholesterol has been proposed to be transported to the liver by HDL particles containing apoE, which may interact with the hepatic remnant and LDL receptors (Eisenberg et al., 1984).

Atherosclerosis is a disease of large and medium-sized muscular arteries and the elastic arteries, such as the aorta and iliac vessels. The basic lesion-the atheroma or fibrofatty plaque consists of a raised focal plaque within the intima, having a core of lipid (mainly cholesterol, usually complexes to proteins, and cholesterol esters) and a covering fibrous cap. This plaque gradually blocks the arteries causing infarction of the tissues (Packer and Landvik, 1989). Several clinical and epidemiological studies indicate that diabetes mellitus is an independent risk factor for CVD. Both type 1 and type 2 diabetes mellitus are associated with a marked increase in risk of CHD, cerebrovascular, and peripheral vascular disease, and these disorders are major causes of morbidity and mortality in diabetes. In general, atherosclerosis and its complications occur earlier and are two to six times more frequent in diabetics than in nondiabetics, and that 80% of all type 2 diabetics will die of an atherosclerotic event. In most studies the excess risk of CHD caused by diabetes is relatively greater in diabetic women than in diabetic men (Ruderman et al., 1992; Eastman et al., 1997; Kannel and McGee, 1979; Garber, 1995). There is debate about the relative roles of hyperglycemia and dyslipidemia in the excess CAD associated with diabetes.
(Eschwege et al., 1994). Whereas, the Diabetes Control and Complications Trial (DCCT) indicated that blood glucose is highly predictive of microvascular disease (DCCTRG, 1993), the contribution of all the commonly measured risk factors can explain no more than 25% of the excess macrovascular CAD associated with diabetes (Pyorala et al., 1987). Importantly, plasma lipoprotein profile may be most critical (Bierman, 1992; Stamler, 1987; Steiner, 1994) because at any total cholesterol level, diabetics have 3- to 5-fold higher CAD mortality rates than do nondiabetic individuals (Stamler, 1987). There is virtually uniform agreement that the diabetic dyslipidemia in type 1 and type 2 diabetes should be highly atherogenic (Ruderman et al., 1992; Semenkovich and Heinecke, 1997; Bierman, 1992). However, underlying mechanisms for the accelerated atherogenesis in diabetes have remained poorly understood. Atherosclerosis vascular disease (ASVD) complications occur more often in patients with type 2 diabetes than in patients with type 1 diabetes. This is at least partly due to the fact that in type 1 diabetes, macrovascular disease usually appears only in the presence of renal complications, whereas in type 2 diabetes, atherosclerotic complications are already present at the time of diagnosis of diabetes.

Several harmful effects of hyperglycemia are known which may contribute to atherothrombosis in type 2 diabetic patients. The onset of hyperglycemia in type 2 diabetic patients with the metabolic syndrome, which is characterized by increased production of free radicals, appears to accelerate atherogenesis, possibly by enhanced formation of glycosylated proteins and advanced glycation products and/or by increasing endothelial dysfunction (Grundy et al., 1999; Maxwell and Lipd, 1997). Consistent with these findings, in patients with type 2 diabetes, the total plasma antioxidant capacity, susceptibility of LDL-C to oxidation and the excretion of oxidized products of arachidonic acid are all increased in proportion to the severity of hyperglycemia (Grundy et al., 1999; Maxwell and Lipd, 1997; Ceriello et al., 1997; Peuchant et al., 1997). Due to increased susceptibility of LDL to oxidation in these hyperglycemic patients, Sobenin et al. (1996) demonstrated that sera from type 2 diabetic patients show increased atherogenic properties, as measured by the ability of LDL-C to accumulate in human aortic intimal cells. Many trials have been conducted to see the effect of correction of hyperglycemia on micro and macrovascular diseases. The recent trial, UKPDS was published in 1998 and showed a 12% reduction in any
diabetes related end point (microvascular and macrovascular complications), a 10% reduction in any diabetes related death, a 6% reduction in all cause mortality, a 16% reduction in myocardial infarction and a 25% reduction in microvascular complications, among intensively treated patients (HbA1c = 7) than with conventionally treated patients (HbA1c = 7.9) for hyperglycemia (UKPDS 33, 1998). Thus, it seems that type 2 diabetes may be a significant risk factor for diabetic macrovascular disease but intensive glucose control reduces only microvascular complications effectively but not macrovascular disease for which aggressive treatment of other risk factors like hypertension, dyslipidemia and smoking are also necessary.

Modified lipoproteins, particularly different forms of oxidized LDL, have been reported to elicit humoral immune responses in both experimental animals and humans. In diabetes, the effects of glycation and oxidation are interwoven, and increased glycation results not only in increased susceptibility of LDL to oxidation but also in increased formation of glycoxidation products or AGEs. Modified LDL triggers the formation of autoantibodies, and both modified LDL and antibodies against modified LDL have been detected in circulation and in atheromatous plaques. Also, ICs containing modified LDL have been isolated from the serum of diabetic and nondiabetic patients with manifestations of atherosclerosis. In addition, it has been demonstrated that in vitro formed LDL-IC and IC isolated from patients are taken up mainly through Fcr receptors and cause intracellular accumulation of cholesterol esters in macrophages and smooth muscle cells.

1.4 Lipoprotein Metabolism

Defects in the lipid and lipoprotein metabolism that is dyslipidemia or atherogenic dyslipidemia is more common in type 2 diabetes than in type 1 and occurs early even at the prediabetic stage of impaired glucose tolerance (Laakso and Barret-Canner, 1995). Excess risk of CHD in patients with diabetes mellitus is not fully understood, but diabetic dyslipidemia is likely to be major contributor (Haffner, 1998). Patients with type 2 diabetes have increased levels of total and VLDL triglycerides, decreased levels of HDL-C and smaller absolute elevations of LDL-C levels relative to nondiabetic patients (Haffner, 1998). However, patients with type 2
diabetes tend to have total LDL-C values that do not meet the goal by National Cholesterol Education Programme Adult Treatment Panel II criteria (Alexander et al., 1998), and have a greater preponderance of smaller, denser LDL-C (sd-LDL-C) that seems more atherogenic (Haffner, 1998). Because of greater preponderance of sd-LDL, a moderately high LDL-C (between 130 and 160 mg/dl) in a type 2 diabetic patient is equivalent to much higher LDL-C in terms of CHD risk for a nondiabetic subjects (Grundy et al., 1999; Haffner, 1998; NCEP Expert Panel, 1993). HDL-C also shows quantitative and qualitative changes in type 2 diabetes. Quantitatively type 2 diabetes is associated with low HDL-C, particularly HDL2-C subfraction along with low apoprotein A1 concentration (Laakso et al., 1985; Garg and Grundy, 1990; Howard, 1994). These abnormalities in HDL persist despite good glycemic control of type 2 diabetes (Chen et al., 1987). The reason for decrease in HDL-C in type 2 diabetes include reduced transfer of surface components to HDL during catabolism of triglyceride rich particles, increased activity of hepatic lipase and increased catabolism of apoprotein A1 (Golay et al., 1987; Bayes et al., 1991; Kasim et al., 1987).

Overall, 30-40 % of patients with diabetes have TG levels >200 mg/dl, and 10 % have TG >400 mg/dl (Cowie and Harris, 1995). However, in the UKPDS, despite a high frequency of modestly elevated base line TG levels (mean base line 159 mg/dl), a multivariate analysis showed that TG levels did not predict CHD events. LDL-C was the strongest independent predictor of CHD followed by HDL-C (Turner et al., 1998), supporting current national guidelines in which LDL lowering is the primary lipid target. Low density lipoproteins (LDLs) are composed of distinct subspecies that differ in size, density, chemical composition, and their association with CVD (Austin et al., 1990; Stampfer et al., 1996; Gardner et al., 1996; Lamarche et al., 1997; Austin et al., 1988). Lipoprotein profiles that are relatively rich in smaller, more dense LDL particles (sd-LDL) are associated with up to 3-fold greater risk of myocardial infarction (MI), than those mainly consist of large buoyant LDL (Ib-LDL) particles (Austin et al., 1990; Stampfer et al., 1996; Gardner et al., 1996; Lamarche et al., 1997; Austin et al., 1988). The increased risk of the sd-LDL phenotype is in part due to associated metabolic aberrations, including high TG and low HDL concentrations (Austin et al., 1990). However, the mass of sd-LDL is independently associated with
disease risk (Stampfer et al., 1996; Gardner et al., 1996; Lamarche et al., 1997; Austin et al., 1988), suggesting that these particles are more directly atherogenic than \( \text{Ib-LDL} \). It has been shown that not only the prevalence of \( \text{sd-LDL} \) but also its concentration was substantially increased in type 2 diabetic patients with and without CHD, as well as subjects with various types of hyperlipidemia (Hirano et al., 2004).

Since \( \text{sd-LDL} \) is generated from large triglyceride rich VLDL, several findings also suggest that subjects with the predominance of \( \text{sd-LDL} \) have higher TG and lower HDL-C and HDL\(_2\)-C concentrations, similar to the levels seen in subjects with insulin resistance and are hyperinsulinemia (Tribble et al., 2001; Austin and Edwards, 1996). Therefore, it appears that there is a close association of \( \text{sd-LDL} \) with the insulin resistance (Berneis and Krauss, 2002). It has been suggested that in comparison to \( \text{Ib-LDL} \), \( \text{sd-LDL} \) are highly atherogenic as a result of their enhanced susceptibility to oxidative modification, higher penetration in the arterial wall, their lower binding affinity for the LDL-receptor, and prolonged plasma half life (Chapman et al., 1998; Bjornheded et al., 1996). In contrast with native LDL, oxidized LDL particles initiate a series of events, including vascular inflammation and macrophage foam cell formation, that are central to the atherogenic process (Tribble, 1999). LDL particles with greater tendency to become oxidized might thus be more likely to participate in proatherogenic events. Differences in oxidative susceptibility between \( \text{Ib-LDL} \) and \( \text{sd-LDL} \) have been attributed to differences in their physicochemical properties. Relative to \( \text{Ib-LDL} \), \( \text{sd-LDL} \) have a reduced content of antioxidants (De Graaf et al., 1991; Tribble et al., 1992; Dejager et al., 1993; Tribble et al., 1994; Tribble et al., 1995), and free cholesterol (De Graaf et al., 1991; Tribble et al., 1992; Tribble et al., 1994), and are enriched with polyunsaturated fatty acids (De Graaf et al., 1991), and, possibly, hydroperoxides (Sevanian et al., 1996). Antioxidant concentrations and free cholesterol content have been shown to predict differences in the oxidative susceptibility of LDL density subfraction (De Graaf et al., 1991; Tribble et al., 1992; Tribble et al., 1994). Lipoproteins (VLDL and LDL) from diabetic rats are more extensively oxidized \textit{in vivo} and more toxic to certain cell lines than LDL from nondiabetic rats (Morel and Chisolm, 1989). A related finding was that correction of hyperglycemia with insulin therapy or supplementation with antioxidants decreased the extent of oxidation and cytotoxicity of diabetic rat lipoprotein (Morel and Chisolm, 1989). Increased rates of lipid peroxidation in type 2 diabetics may be
related in part to increased amounts of sd-LDL in their plasma (Feingold et al., 1992; Selby et al., 1993). Based on the above information, sd-LDL has been highlighted as a useful and potent new marker for the risk of CHD or type 2 diabetes (Austin et al., 1995).

1.4.1 Atherogenic properties of oxidized LDL

The etiology of CVD is complex and multifactorial, but there is substantial evidence that oxidized lipoproteins play an important role in the pathogenesis of atherosclerosis (Diaz et al., 1997). It has been shown in rodents (Staprans et al., 1996) and humans (Staprans et al., 1994). Oxidized fatty acids in the diet are incorporated into chylomicrons. In rodents, oxidized fatty acids are also incorporated into the endogenous serum VLDL+LDL fraction (Staprans et al., 1993). They have also demonstrated that the levels of oxidized fatty acids in the circulation correlate directly with the quantity of oxidized fatty acids in the diet and that oxidized fatty acids accelerate atherosclerosis in rabbits (Staprans et al., 1996). Staprans et al. (1998), have demonstrated that oxidized cholesterol in the serum of rabbits is both produced endogenously, and derived from food. After feeding rabbits a diet containing nonoxidized cholesterol that contains no detectable levels of oxidized cholesterol, cholesterol oxidation products were identified in serum VLDL and LDL. Feeding a diet enriched in oxidized cholesterol (Staprans et al., 1998), or injections of oxidized cholesterol (Rong et al., 1999), to cholesterol-fed rabbits resulted in a 2-fold increase in fatty streak lesions in the aorta (Zainuddin, 2004; Beg and Zainuddin, 2003). Therefore, dietary oxidized cholesterol may be a risk factor for atherogenesis. Cholesterol oxidation products are abundant in oxidatively modified LDL (Hodis et al., 1991) and high levels of circulating cholesterol oxidation products are found in rabbits fed a cholesterol containing diet (Hodis et al., 1991; Hodis et al., 1992). However, direct in vivo evidence linking circulating cholesterol oxidation products to early vascular lesion formation is only recently becoming available (Staprans et al., 1998; Rong et al., 1999; Zainuddin, 2004; Beg and Zainuddin, 2003). These findings are supported by another report indicating that relative to normolipidemic LDL, LDL from homozygous familial hypercholesterolemic subjects exhibit distinctive physicochemical properties and biological activities that may contribute to initiation and progression of atherogenesis in vivo (Yang et al., 2003). Patients with diabetes
mellitus have a high incidence of CHD in comparison to nondiabetic CHD patients (Uusitupa et al., 1990). In light of this, the NCEP Expert Panel suggests that the goal of lowering LDL cholesterol (i.e. <100 mg/dl) in diabetic patients should be the same as subjects with CHD (EPDETHBCA, 1993). Moreover, the diabetic state is characterized by an increased rate of lipoprotein oxidation, which may play an important role in the development of atherosclerosis (Reaven, 1995). Diabetes can affect lipoprotein metabolism in multiple ways. In diabetic subjects, numerous potential mechanisms were able to mediate premature atherosclerosis. It has been well documented that diabetic patients have increased glycation and increased lipoprotein oxidation (Bellomo et al., 1995) and reduced antioxidant status (Maxwell et al., 1997). Thus, oxidative modification of lipoproteins should be one of the mechanisms for an early development of atherosclerosis in diabetics. Oxidation of LDL is initiated by both enzyme mediated and nonenzymic mechanism in vivo, and oxidized LDL has many atherogenic properties. Oxidation of LDL in vivo is likely to be influenced by the local environmental factors such as pH. The composition of LDL is also important, including such factors as antioxidant content, fatty acid composition and particle size. Oxidized LDL (Ox LDL) has many characteristics that potentially promote atherogenesis, in addition to the ability to be taken up rapidly by macrophages to form foam cells. It is chemoattractant for circulating monocytes (Quinn et al., 1987), both directly and also via stimulation of the release of monocyte chemoattractant protein-1 from endothelial cells (Cushing et al., 1990). The chemoattractant activity of LDL resides in its lipid moiety and is attributable to lysophosphatidylcholine generation during the conversion of LDL in to its oxidized form. Ox LDL promotes the differentiation of monocytes in to tissue macrophages by enhancing the release of macrophage colony stimulating factor from endothelial cells (Rajavashisth et al., 1990), and inhibits the motility of resident macrophages (Quinn et al., 1987). It is chemoattractant for T-cells (McMurray et al., 1993), although not for β-cells, and consequently the atherosclerotic plaque contains primarily monocytes and T cells. Unlike native LDL, Ox LDL is immunogenic (Palinski et al., 1989), and it is also cytotoxic to various cell types including endothelial cells (Hessler et al., 1983), resulting in loss of endothelial integrity. It inhibits tumor necrosis factor expression (Hamilton et al., 1990), stimulates release of interleukin-1β (Thomas et al., 1994) from monocyte/macrophages, and can inhibit endothelial cell-dependent
arterial relaxation (Ohgushi et al., 1993). Ox LDL also activates matrix-digesting enzymes, which may play a role in plaque instability (Xu et al., 1999). However, some cellular responses to peroxidation products appear to be protective, and it is possible that peroxidation is an essential intermediary in an effective response to an oxidative insult (Parthasarathy et al., 1999).

1.4.2 Occurrence and mechanism of LDL oxidation in vivo

Several lines of research support the occurrence of LDL oxidation in vivo (Steinberg, and Lewis, 1997). Oxidized apo B-100 epitopes and increased levels of lipid peroxidation products can be detected in LDL extracted from both rabbit and human atherosclerotic lesions (Yla-Herttuala et al., 1989). Immunohistochemical staining of atherosclerotic lesions with specific monoclonal antibodies has demonstrated the presence of Ox LDL (Palinski et al., 1989). Circulating anti-OxLDL antibodies have been demonstrated in serum, and titers correlate with the progression of atherosclerotic lesions (Palinski et al., 1989). Several studies in different animal models of atherosclerosis strongly suggest that progression of the lesions can be delayed by intervention with antioxidants (Steinberg, and Lewis, 1997). The fact that several different antioxidants such as, probucol, vitamin E, butylated hydroxytoluene and diphenylphenylenediamine have been used, supports the implication that the anti-atherogenic effect is due to the antioxidant properties of these drugs, rather than any other biological effect (Rajavashisth et al., 1990). Epidemiological evidence, including ecological, case-control and prospective studies, indicates low antioxidant consumption is associated with an increased risk of CVD (Gey and Puska, 1989; Rimm et al., 1993). The evidence is strongest in case of vitamin E, with less consistent support for the protective roles of vitamin C, carotenoids and flavonoids (McMurray et al., 1993). Several trials of antioxidant supplementation in patients at risk of vascular events have demonstrated a reduction in cardiovascular end points (Blot et al., 1995; Stephens et al., 1996; Gruppo Italino per lo Studio della Sopravvivenzanell’ Infarto miocardico1999; Salonen et al., 2000; Yusuf et al., 2000), while other studies have been negative (The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group, 1994; Hennekens et al., 1996; Redlich et al., 1999; Walldius et al., 1993). The discrepancies in these trials may reflect differences in the dose and
mix of antioxidant used as supplements, the different dietary backgrounds of the subjects and intervention at relatively advanced stage of disease.

Many cell types are capable of oxidizing LDL, including monocytes, macrophages, neutrophils, endothelial cells, smooth muscle cells and fibroblasts. However, cell types that are involved in the atherosclerotic lesion in which OX LDL is found, i.e. macrophages, endothelial cells and smooth muscle cells, would seem to be the most likely to contribute to LDL oxidation in vivo. It appears likely that LDL is oxidized in microdomains in the arterial walls sequestered by proteoglycans and other extracellular matrix constituents, where it is protected from plasma antioxidants (Carmena et al., 1996). It is still unclear which oxidative mechanisms or radical species are involved; potential candidates include NADPH oxidase, myeloperoxidase, cytchrome P₄₅₀, the mitochondrial electron transport chain, peroxynitrite, xanthine oxidase, ceruloplasmin and lipoxygenase. The last enzyme has received much attention with the discovery that lipoxygenase modify LDL in vitro to a form taken up by the scavenger receptor (Steinberg, 1999). In addition, disruption of the lipoxygenase gene diminishes atherosclerosis in transgenic mice (Cyrus et al., 1999). Once initiated, oxidation of LDL is a free radical driven lipid peroxidation chain reaction. Lipid peroxidation is initiated by free radical attack on double bond associated with a polyunsaturated fatty acid (PUFA). This results in the removal of a hydrogen atom from a methylene (CH₂) group, the rate of which determines the rate of initiation, a key step in lipid peroxidation. Molecular rearrangement of the resulting unstable carbon radical results in a more stable configuration, a conjugated diene. The conjugated diene reacts very quickly with molecular oxygen, and the peroxy radical thus formed is a crucial intermediate (Abuja and Esterbauer, 1995) (Fig. 1.6). A PUFA peroxy radical in LDL may abstract a hydrogen atom from an adjacent PUFA to form a hydroperoxide and another lipid radical, a reaction which results in chain propagation. Removal of hydrogen atoms by the peroxy radical from other lipids, including cholesterol, eventually yields oxysterols. Lipid hydroperoxides fragment to shorter-chain aldehydes, including malondialdehyde and 4-hydroxynonenal. These reactive aldehydes in turn may bind to ε-aminogroups of apo B-100, giving the protein an increased net negative charge. The classical LDL receptor recognizes a specific domain of positive charges from lysine, arginine and histidine residues on
Fig. 1.6. Basic reaction sequence of lipid peroxidation.
apo B. Alteration of this domain results in failure of binding by the apo B/E receptor and an increase in negative surface charge on apo B-100 results in increased recognition by the scavenger receptor.

In the presence of a lipid phase chain breaking antioxidant such as α-tocopherol, the peroxyl radical may be scavenged. The tocopheroxyl radical thus formed has very low reactivity and will generally result in chain termination. LDL exposed to oxidative stress in vitro will not form significant amounts of hydroperoxides until it becomes depleted of chain breaking antioxidants. In vitro studies of LDL oxidation have verified the existence of “lag phase”, during which significant oxidation of LDL can not be detected, prior to the onset of the “propagation phase”, presumably after the endogenous antioxidants have been consumed. A steady increase in the detectable byproducts of oxidation continues, until the substrate, i.e. the PUFAs, has been depleted, and a plateau phase is reached. Assessment of resistance of LDL to oxidation has generally involved in measurement of the duration of the lag phase including other variables, such as the rate of propagation, are also noted.

1.4.3 Factors influencing susceptibility of LDL to oxidation

The susceptibility of LDL to oxidation in vivo is influenced by both LDL composition (intrinsic factors) and the microenvironment in which the LDL is found (extrinsic factors). Among the intrinsic factors, the fatty acid composition of LDL is of prime importance; a high proportion of PUFAs confers greater susceptibility to oxidation, while a high proportion of monounsaturated fatty acids (MUFAs) protects against oxidation (Reaven et al., 1991; Reaven et al., 1993; Thomas and Rudel, 1996). Since the propagation phase of LDL oxidation begins after the endogenous antioxidants have been consumed, susceptibility to oxidation is also highly dependent on the antioxidant content, which in lipoproteins is mainly α-tocopherol, although ubiquinol-10 and carotenoids are also important (Stocker et al., 1991). The molar ratio of PUFA to total antioxidants in LDL is approximately 150: 1. Since there is considerable variation in dietary fatty acid content between subjects, lipophilic antioxidant intake also varies significantly from individual to individual (Klatt and Esterbauer, 1996). Dietary supplementation with vitamin E results in increased LDL
resistance to copper-induced oxidation in vitro (Reaven and Witztum, 1993; Wiseman et al., 1995; Suzukawa et al., 1995; Ziouzenkova et al., 1996), an effect i.e. is dose dependent. LDL size is another factor that has been shown to affect oxidative susceptibility (Chait et al., 1993). The small, dense subfractions of LDL are more susceptible to oxidation than large, less dense LDL particles, which to a large extent may be due to differences in antioxidant content (Tribble et al., 1994). A preponderance of sd-LDL particles is found in subjects with moderately elevated triglycerides (1.5 mM), and is due to the metabolism of relatively large lipid rich VLDL particles. The concentration of preexisting fatty acid peroxides in LDL also exert an effect on the oxidative susceptibility of the particle, with higher levels of these peroxides being associated with a shorter lag time when transition metals are used to initiate oxidation (O'Leary et al., 1992).

1.5 Antiatherogenic Functions of High Density Lipoproteins

Epidemiological studies have identified low density lipoproteins and high density lipoproteins as independent risk factors that modulate CVD risk. The role of HDLs in protecting against the development of CHD has been demonstrated in numerous clinical studies. The studies show an inverse relationship between the concentration of HDL-C and the development of premature CHD (Castelli et al., 1992; Gordon and Rifkind, 1989; Gordon et al., 1989; Assman et al., 2004), with a reduction in CHD risk of 2-4 % for each 1 mg/dl increase in HDL-C. HDL is a class of heterogeneous lipoproteins containing approximately equal amounts of lipid and protein (Gordon and Rifkind, 1989). HDL particles are characterized by high density and small size. The various HDL subclasses vary in quantitative and qualitative content of lipids, apolipoproteins, enzymes, and lipid transfer proteins, resulting in differences in shape, density, size, charge, and antigenecity. Apo-A1 is the predominant HDL protein followed by apo-A2. HDL fraction accounts for almost all of the cholesterol quantified as HDLC. HDL can be further fractionated by density into HDL2 and HDL3, by size, or by apolipoprotein composition. The origin of HDL particles is not entirely clear. Several mechanisms have been proposed, including direct secretion into plasma from hepatocytes or enterocytes; release during the interconversion of various HDL subpopulations by phospholipid transfer protein (PLTP), cholesteryl ester transfer protein (CETP), or hepatic lipase (HL); or direct
interaction of free lipoproteins with cell membrane (von Eckardstein et al., 2000). Although our understanding of how HDL protects against CVD is still incomplete, there is evidence that supports at least three major atheroprotective mechanisms of HDL:

1.5.1 HDL-mediated cholesterol efflux

The reverse transport of cholesterol from peripheral cells to sites of catabolism, first described by Glomset and Norum in 1973, has been suggested to be the primary antiatherogenic mechanism of HDLs. Cholesterol efflux from macrophages to HDL can occur by passive diffusion (Yancey et al., 2003), by interaction with the SR-B1 receptor (Williams et al., 1999), or by binding to the ABCA1 transporter (Rubin et al., 1991; Takahashi and Smith, 1999; Remaley et al., 2001; Oram and Lawn, 2001; Liu et al., 2003) (Fig. 1.7). The preferred acceptor for the ABCA1 transporter-mediated cholesterol efflux is poorly lipidated apo-A1 (Castro and Fielding, 1988), which is converted to spherical α-HDL after esterification of free cholesterol to cholesteryl esters by LCAT. Both the SR-B1 (Malerod et al., 2002) and ABCA1 transporter (Costet et al., 2000; Schwartz et al., 2000; Venkateswaran et al., 2000) pathways are modulated by the cellular content of oxysterols, which regulate the LXR pathway and expression of the SR-B1 and ABCA1 transporter genes (Fig. 1.7). After accepting excess cellular cholesterol from arterial macrophages and other peripheral tissues HDL transports the excess cholesterol to the liver for disposal. HDL is thus an integral component of the atheroprotective reverse cholesterol transport process, functioning as a carrier of excess cellular cholesterol from peripheral tissues to the liver, where it is excreted from the body as bile acids and cholesterol (Glomset, 1968).

1.5.2 HDL and endothelial function

The second mechanism by which HDL may protect against CVD is the selective decrease of endothelial cell adhesion molecules, which facilitate the binding of mononuclear cells to the vessel wall and promote lesion development (Barter et al., 2002). The HDL-induced increase in NO production may be an important atheroprotective feature of HDL, as diminishing bioavailability of endothelial-derived NO has a key role in the early pathogenesis of hypercholesterolemia-induced vascular
Fig. 1.7. Mechanism of action of HDL as an antioxidant via three major pathways.  
disease and atherosclerosis (Cohen, 1995; Naruse et al., 1994; Cayatte et al., 1994). Apoptosis of endothelial cells has been demonstrated in atherosclerosis. The ability of HDL to inhibit apoptosis has been shown in several studies (Nofer et al., 2001; Sugano et al., 2000). HDL exerts a protective effect by interfering with the apoptotic stimuli which endothelial cells are exposed. The mechanism is uncertain but this HDL function may contribute to longevity.

1.5.3 HDLs and antioxidative mechanisms

A third mechanism by which HDL decreases atherosclerosis is to protect LDL from oxidation. Oxidized or modified LDL, unlike normal LDL, is readily taken up by the scavenger receptor SR-A or CD36 on macrophages, resulting in cholesteryl ester accumulation with foam cell formation. The cholesterol-loaded macrophage produces a number of inflammatory cytokines and stimulates MCP-1 as well as endothelial cell adhesion molecules. Mechanisms of LDL oxidation in vivo involve concerted modification by chemically diverse oxidants, employing that any single low molecular weight antioxidants, such as vitamins E and C, even at physiologically relevant doses, may not provide complete oxidative protection of LDL in vivo (Witztum and Steinberg, 2001; Gaut and Heinecke, 2001). Plasma HDLs possess a spectrum of antiatherogenic actions, including potent antioxidant and anti-inflammatory activities (Van Lenten et al., 2001). Although HDLs can themselves undergo oxidative modification (Francis, 2000), several enzymes that may cleave oxidized lipids and thereby inhibit LDL oxidation are associated with HDL particles; these include paraoxonase (PON) in its major isoforms PON1 (Durrington et al., 2001), platelet-activating factor acetylhydrolase (PAF-AH) (Tselepis and Chapman, 2002), LCAT (Goyal et al., 1997), and glutathione selenoperoxidase (Chen et al., 2000). In addition, apo-A1, a major HDL apolipoprotein can remove oxidized lipids from LDL, suggesting that HDL can function as an acceptor of oxidized lipids (Navab et al., 2000). Apo-A1 was shown to reduce peroxides of both phospholipids and cholesteryl esters and to remove hydroperoxides, which are products of 12-lipoxygenase, from native LDL (Navab et al., 2000). Other HDL apolipoproteins, such as apo-AII (Boisfer et al., 2002), apo-AIV (Ostos et al., 2001), apo-E (Miyata and Smith, 1996), and apo-J (Kelso et al., 1994), also function as antioxidants in vitro.
The diversity of antioxidative actions of HDL particles suggests that HDL provide efficient protection of LDL from oxidation in vivo. Plasma LDLs are heterogeneous in their physico-chemical properties and consist of three major particle subclasses, Id-LDL, intermediate LDL, and Sd-LDL; such LDL subfractions are distinct in their atherogenic and oxidative properties (Chapman et al., 1998; Kontush et al., 2003). Similarly, circulating HDL particles are heterogeneous in physico-chemical properties, intravascular metabolism, and biological activity (von Eckardstein et al., 1994; Lamarche et al., 1999). Ultracentrifugally isolated HDL3 exerts greater inhibition of adhesion protein expression in endothelial cells than HDL2 (Ashby et al., 1998). Isopycnic density gradient centrifugation allows reproducible isolation of 5 physicochemically defined, highly purified, major HDL subfractions, HDL2b, 2a, 3a, 3b, and 3c (Chapman et al., 1981; Goulinet and Chapman, 1997). Indeed, HDL particle phenotypes are qualitatively and quantitatively altered in dyslipidemias associated with premature atherosclerosis, including hyperlipidemias of types IIA, IIB, and IV, and type 2 diabetes (Lamarche et al., 1999). Significant differences between the HDL fractions derived from subjects with type 1 diabetes and those derived from control subjects has been reported (Valabhji et al., 2001). The greater values for HDL esterified cholesterol and phospholipid in the subjects with type 1 diabetes are attributable largely to increased concentrations of esterified cholesterol and phospholipid associated with larger, more buoyant HDL fractions (Valabhji et al., 2001). Paraoxonase catalyzes the break down of oxidized phospholipids in LDL. Studies have found that transgenic animals deficient in this enzyme are significantly more susceptible to the development of diet-induced atherosclerosis (Shih et al., 1998; Shih et al., 2000). On the other hand, PON transgene in mice produces HDL resistant to oxidation (Oda et al., 2002). Degradation of oxidized phospholipids has also been attributed to PAF-AH. Over expression of human apo-A1 in apo-E knockout mice increases PAF-AH activity and simultaneously reduces oxidative stress in plasma, decreases intracellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM) expression, and decreases monocyte recruitment in to the arterial wall (Theilmeier et al., 2000).

Serum paraoxonase is a calcium-dependent esterase that is known to catalyze hydrolysis of organophosphates, and is widely distributed among tissues such as liver,
kidney, intestine, and also serum, where it is associated with HDL (La Du, 1996; Mackness et al., 1996). PON specificity towards endogenous serum and tissue substrates is not well characterized and therefore synthetic substrates, such as paraoxon and phenyl acetate are used to monitor the enzyme's activity. Serum PON activity was shown to be reduced in patients after MI (Ayub et al., 1999), in patients with familial hypercholesterolemia (Mackness et al., 1991), and in patients with diabetes mellitus (Mackness et al., 1991; Abbott et al., 1995), diseases that are associated with accelerated atherogenesis. Genetic variations of PON correlated with HDL-cholesterol and apo-A1, suggesting antiatherogenic properties. Atherosclerosis occurs naturally in humans and rabbits but not in mice. Comparison of PON arylesterase activity revealed that in humans and rabbits, > 95 % is HDL associated. In mice, about 30 % of PON activity is lipid poor; in the absence of apo-A1 in mice, total PON arylesterase activity is reduced and > 60 % is lipid poor. It is striking that the rabbit has so much higher level of plasma PON activity than human and mice, especially in view of the fact that out of these three species, the rabbit is the most susceptible to cholesterol-induced atherosclerosis (Cabana et al., 2003). The PON activity is much more labile to the acute phase reaction in the rabbit than in the mouse, resulting in a substantial decrease in rabbits treated with croton oil (Cabana et al., 2003). Feingold et al. (1998) have demonstrated that administration of endotoxin lipopolysaccharide in Syrian hamsters was associated with a decline in serum PON concentration and activity as well as PON mRNA levels in the liver. A decrease in PON activity during the acute phase response could therefore be another factor linking the acute phase response with increased atherogenesis (Feingold et al., 1998). Boemi et al. (2001) have demonstrated that lower absolute PON concentrations and activities, higher LDL: PON concentration ratios and a lesser capacity to prevent LDL oxidation are consistent with the hypothesis that reduced serum PON in type 1 patients, similar to type 2 diabetics, could contribute to increased risk of vascular disease. Although PON can offer protection against the toxicity of some organophosphates, its physiological role is still not clear; however, evidence exists for a protective effect of PON against oxidative damage (Mackness et al., 1996). PON was suggested to contribute to the antioxidant protection conferred by HDL on LDL oxidation (Mackness and Durrington, 1995; Mackness et al., 1991; Navab et al., 1996). The effect of HDL-associated PON or of purified PON on the LDL oxidation
process, including its initiation (conjugated diene formation), propagation (peroxides formation), and decomposition (aldehydes formation) phases could be analyzed by using PON inhibitors. The inhibitory effect of HDL on LDL oxidation was suggested to be related to metal ion chelation, or to peroxidase, like activity. Under oxidative stress, not only LDL is susceptible to lipid peroxidation but all other serum-lipids, including those present in HDL, are also prone to oxidation. In fact, HDL has been shown to be the major carrier of lipid hydroperoxides in human serum (Bowry et al., 1992; Hahn and Subbiah, 1994). In this context it is interesting to mention that HDL-associated cholesteryl ester hydroperoxides are more rapidly reduced to their less reactive hydroxides than are those associated with LDL (Christison et al., 1995). Oxidative modification of HDL has also been shown to impair the ability of the lipoprotein to promote cholesterol efflux (Nagano et al., 1991; Morel, 1994). Thus, inhibition of HDL oxidation by PON may preserve the antiatherogenic functions of HDL in reverse cholesterol transport, as well as its protection of LDL from oxidation. Kontush et al. (2003) have demonstrated that both serum and plasma-derived small, dense HDL particles possess the most potent capacity among HDL subspecies to protect LDL from both metal-dependent and metal-independent oxidation in normolipidemic subjects. The oxidative protection of LDL by ultracentrifugally isolated HDL subfractions (at equal cholesterol or protein concentration or equal particle number) increased in the order HDL2b < HDL2a < HDL3a < HDL3b < HDL3c. HDL subfractions efficiently protected not only total LDL but also intermediate LDL3 (typically the most abundant LDL subfraction in normolipidemic subjects) and small, dense LDL5 (a highly atherogenic LDL subfraction) (Chapman et al., 1998), thereby suggesting that HDL can attenuate oxidation of atherogenic LDL subclass. When HDL subfractions were subjected to nonmetal or copper-induced oxidation in the absence of LDL, their oxidative resistance increased in the order HDL2b < HDL2a < HDL3a < HDL3b < HDL3c, thereby mirroring their antioxidative activity during LDL oxidation. Similarly, PON1 activity with phenyl acetate or paraoxon as substrate increased in the order HDL2b < HDL2a < HDL3a < HDL3b < HDL3c (Kontush et al., 2003). On a particle basis, contents of apo-A1 and apo-A2 were elevated in HDL3a and lowest in HDL3c. PAF-AH activity was significantly increased in small, dense HDL3c. LCAT activity was higher in HDL3 relative to HDL2 subfractions (Kontush et al., 2003). The present evidence for the differential
antioxidative properties of HDL subfractions may have important consequences for our understanding of the protective antiatherogenic action of HDL in vivo. Thus, although small, dense HDL3c typically accounts for <15 % of total HDL, HDL3c may nonetheless play a pivotal role in the protection of LDL against oxidation, significantly exceeding the protection afforded by low molecular weight antioxidants. Indeed, it has been shown that HDL₃, rather than HDL₂, is strongly correlated with the antiatherogenic action of gemfibrozil in the VA-HIT Study (Robins et al., 2001). Considered together these findings identify small, dense HDL as a potential pharmacological target for the therapeutic attenuation of atherosclerosis in subjects had high cardiovascular risk associated with increased oxidative stress, as, for example, in the case of type 2 diabetes and metabolic syndrome.

1.6 Management of Hyperglycemia/Dyslipidemia in Diabetes Mellitus.

Improving glycemic control in individuals with moderate to severe hyperglycemia regardless of type of treatment is associated with improvement in lipid values. Treatment of hyperglycemia is stepwise and typically dependent on duration of disease. To prevent microangiopathy, neuropathy, and perhaps macrovascular disease, a prudent therapeutic goal is to reduce the glycohemoglobin to ≤ 1 % above the upper limit of normal values (UKPDS, 1998). Weight loss and increased exercise are first-line therapy for reducing hyperglycemia. If hyperglycemia persists, a sulphonylureas or metformin can be used next. Metformin also proved efficacious, although an apparent increase in death rates on the combination of metformin and sulphonylureas has been reported. Another promising group of agents for treatment of type 2 diabetes includes the thiazolidinediones. These agents lower glucose levels by reducing insulin resistance. Troglitazone in combination with insulin therapy is used to improve glycemic control. Unfortunately, troglitazone produces rare but severe liver toxicity (Gitlin et al., 1998). Nonetheless, despite its potential hepatotoxicity, troglitazone is currently being widely used to treat hyperglycemia. New drugs of the same class, rosiglitazone and pioglitazone, may have less potential hepatotoxicity. A different type of drug available for glucose control is acarbose; this agent partially blocks glucose absorption. In patients who failed to achieve glucose control and near normal hemoglobin A₁ levels by changes in life habits and oral hypoglycemic agents, insulin should be initiated. Metformin is the only antihyperglycemic agent that has
been shown to have a beneficial effect on the plasma lipid profile, by mediating a modest reduction in triglyceride levels in hyperlipidemic and hypertensive diabetic patients (Palumbo, 1998). In a head-to-head comparison study (Goldberg et al., 2005), pioglitazone was associated with significant triglyceride reduction, whereas there was no net triglyceride change with rosiglitazone. Although both agents increased HDL-C and LDL-C, pioglitazone was associated with a greater increase in HDL-C and less LDL-C increase than rosiglitazone.

Diet, exercise, and weight loss in overweight individuals are essential in the management of lipid disorders in diabetes. The NCEP and American Diabetes Association (ADA) concur in reducing the intake of saturated and trans-saturated fatty acids to lower LDL-C levels (NCEP ATP III, 2001; ADA, 2003). The NCEP Adult Treatment Panel III (ATP III) recommends limiting the intake of saturated fat to < 7% of daily calories and limiting the intake of cholesterol to < 200 mg per day. Additional dietary options to lower LDL-C include increasing the amount of soluble dietary fiber to 10-25 g daily, adding 2 g daily of plant stanols/sterols, and including soya proteins in the diet. These interventions have been associated with a 5-15% reduction in LDL-C values. The ATP III also recommends limiting the intake of carbohydrates to < 60% in individuals with elevated triglycerides and low HDL-C levels. The ADA also recommends replacing saturated fats with carbohydrate or monounsaturated fat.

Diabetes is considered a CHD equivalent. Therefore, lipid targets for individuals with diabetes are the same as those for individuals with established CHD (NCEP ATP III, 2001). The primary target is an LDL-C < 100 mg/dl. Recently, the NCEP and ATP III lowered the cut point for pharmacological intervention > 130 to > 100 mg/dl and provided an optional lower target of 70 mg/dl for very-high-risk patients, such as those with diabetes and heart disease (Grundy et al., 2004). The second lipid strategy is to increase HDL-C levels, and the third is triglyceride lowering. For individuals with LDL-C levels between 100 and 129 mg/dl, both set of guidelines now support statin therapy to achieve at least a 30-40% LDL-C reduction. In order to achieve a 30-40% LDL-C lowering, at least moderate doses of statin (rosuvastatin 5-10 mg/day, atorvastatin 10-20 mg/day, simvastatin 20-40 mg/day, or pravastatin, lovastatin, or fluvastatin 40-80 mg/day) should be used. Individuals with
diabetes who have CVD should be considered for maximal intensity statin or combination therapy. When the NCEP LDL-C target is not achieved with a statin alone or where statins are not tolerable, combination therapy with etezimibe, bile acid sequesterants, or high dose niacin should be considered. The major clinical concerns with higher doses of statins are liver toxicity and myopathy. Non-HDL-cholesterol is the second therapeutic target according to the ATP III in individuals with TG levels > 200 mg/dl. The therapeutic options for patients with LDL-C < 100 mg/dl (< 70 mg/dl if at a very high risk) on statins to lower non-HDL-C to target (< 130 mg/dl) include combination therapy with fibrate or niacin or alternatively increasing the dose of statin or switching to a more potent statin. Fibrates lower TG levels more efficiently than do statins and might be preferred in individuals with significantly elevated TGs (> 300 mg/dl). Fibrate therapy is the first line of treatment for individuals with TG levels > 500 mg/dl in whom TG lowering is given first priority. The ATP III and the ADA indicate that in high risk patients with HDL-C levels < 40 mg/dl (< 50 mg/dl in women), HDL-C raising should be considered, although neither guide lines defines a target level. Fibric acid derivatives and niacin, the two agents most commonly recommended for HDL raising. It has been clearly shown that the addition of ezetimibe to a statin will lower LDL-C to the designated target than statin alone (Gagne et al., 2002). Bile acid sequestrants may also help to lower LDL-C but should be used with caution because they have a TG raising effect in hypertriglyceridemic patients (Crouse, 1987). It is also clear that achievement of all three lipid goals is more likely with statin plus fibrate or statin plus niacin combinations. Fenofibrate appears to have significantly lower pharmacokinetic interactions with statins compared with gemfibrozil, a consideration to take in to account when using fibrate plus statin combinations. Probucol has been reported to effectively reduce plasma cholesterol in human and a number of animal species (Martz, 1979). It also affects the composition and in vitro catabolism of LDL in Type IIa hypercholesterolemia (Baudet et al., 1986). It increases the activity of plasma lipoprotein lipase and decrease HDL- and LDL-cholesterol concentration in rats. Probucol prevents the development of macrophages into foam cells by inhibiting the lipid storage in macrophages (Yamamoto et al., 1986a). These observations probably accounts for the clinical findings that probucol causes a more marked regression of xanthomas than would be expected from the lowering of LDL-cholesterol levels alone. Probucol
seems to act by increasing LDL removal from the plasma by an LDL receptor independent mechanism (Kasaniemi and Grundy, 1984), as it causes moderate reduction in LDL-cholesterol in non-familial hypercholesterolemia and a smaller decrease in familial hypercholesterolemic patients (Durrington and Miller, 1985; Fellin, 1986). A marked decrease in cholesterol has been constant finding (Kasaniemi and Grundy, 1984; Fellin, 1986) but circulating HDL-cholesterol in probucol treated patients is less than in controls (Yamamoto et al., 1986b), which minimizes the use of probucol as a hypocholesterolemic agent. Omega 3 fatty acids (fish oils) lower TGs with minimal effects on HDL-C and LDL-C levels. They are well tolerated at the commonly used dosages of 1-2 capsules daily (2-4 g/day). At higher dosages there may be worsening of glycemic control. Omega 3 fatty acids are primarily used in the treatment of more marked hypertriglyceridemia usually as second line therapy after a fibric acid derivative. However, they can also be combined with statins.

One of the areas, which have attracted a great deal of attention, is antioxidant nutrition in the control of degenerative diseases such as CVD in diabetics, nondiabetics with established CHD and cancer. Several epidemiological studies have demonstrated an association between increased intake of antioxidant vitamins such as vitamin E (tocopherols and tocotrienols) and vitamin C and reduced morbidity and mortality from CAD. Case-control, and prospective cohort studies have found inverse associations between the frequency of CAD and dietary intake of antioxidant vitamins. Randomized therapeutic trials have thus far shown no benefit with β-carotene and possible benefit with vitamin E (Diaz et al., 1997). Diabetes mellitus is associated with increased oxidative damage of various tissues and organs due to the accumulation of lipid peroxides and AGEs (Brownlee et al., 1984; Lyons, 1992). Dietary supplementation of vitamin E, specifically, tocopherol, has been shown to be effective in reducing the levels of lipid peroxides or AGE in diabetic patients (Ceriello et al., 1991; Frei et al., 1988; Jain, et al., 1996; Rifici and Khachadurian, 1993) and in STZ-induced diabetic rats (Aoki et al., 1992; Vannucchi et al., 1999). Ceriello et al. (1991) have demonstrated that vitamin E administration may reduce protein glycosylation and labile HbA1 in diabetic subjects, independent of changes in plasma glucose. Similar beneficial effects have also been reported for vitamin C. Several studies showed that when pharmacologic doses of vitamin C or E were given
to group of diabetics, there was significant improvement in their glycemic control and insulin action (Paolisso et al., 1993a; Davie et al., 1992; Ceriello et al., 1991; Paolisso et al., 1993b). Supplementation of vitamin C was also associated with normalization of vasodilatory response in patients with hypertension (Taddei et al., 1998) and increased blood flow in type 1 and type 2 diabetic patients (Timmi et al., 1998; Ting et al., 1996). Several reports in poorly control diabetic subjects indicate increased levels of lipid peroxides (Sato et al., 1979), increased LDL susceptibility to oxidation (Tsai et al., 1994; Bonet and Knopp, 1992), and decreased levels of antioxidants (Tsai et al., 1994; Otero et al., 1997; Jain et al., 1991) linking the oxidation of LDL to their accelerated atherosclerotic process. The mechanisms involved in the association between glucose, LDL oxidation, and atherosclerosis is not well understood. In a red blood cell model of oxidative damage, it has been shown that glucose may act either as an antioxidant or prooxidant, depending on the cell concentration of vitamin E (Wang et al., 1996), in a manner similar to shown for vitamin C, dehydroascorbic acid, and flavonoids (Otero et al., 1997; Ma et al., 1994; Stait and Leake, 1994; Wefers and Sies, 1988). Das et al. (2006) have demonstrated that the serum lipid peroxidation level was significantly increased in hypercholesterolemic patients and their LDL has shown a greater propensity towards in vitro oxidation. Hypercholesterolemic LDL required a higher amount of ascorbic acid to reduce its oxidation level as compared to LDL isolated from normocholesterolemic individuals. These compounds may act either as LDL antioxidant or prooxidant, depending on the LDL vitamin E content, when vitamin E is present in the LDL those compounds act as antioxidant, whereas when during the process of LDL oxidation, once vitamin E is consumed (Otero et al. (2002) or to minimally oxidized LDL (Ma et al., 1994; Stait and Leake, 1994), they act as prooxidant, accelerating the oxidation of LDL. It has been reported by Otero et al. (2002) that incubation of purified human LDL with glucose was associated with delaying the early phases of copper-mediated LDL oxidation, slowing the vitamin E consumption, but it accelerates the rate of LDL oxidation once LDL associated vitamin E has been consumed, these effects were concentration dependent (Otero et al. (2002). These results also demonstrated that the concentration-dependent prooxidant effect of glucose is in agreement with the fact that type 1 diabetics with complications secondary to diabetes are related to the degree of metabolic control, those with the highest levels of glucose have the highest
risk of developing atherosclerosis and CVD (DCCTRG, 1993; Richard et al., 1993). In addition, the data also indicate (Otero et al., 2002) that increasing the vitamin E content of the LDL, the glucose prooxidant effects are markedly reduced. In contrast, the diabetic subjects with the lowest intake of vitamin E, and therefore, lower levels of vitamin E in their LDL would be more susceptible to the effects of the hyperglycemia. These results are consistent with earlier studies in type 1 diabetic patients with lower levels of vitamin E (Otero et al., 1997; Jain et al., 1991), making them more susceptible to the prooxidant effects of the hyperglycemia. Several studies in the animal model have shown that some of the complications secondary to diabetes can be prevented with the administration of antioxidants including vitamin E (Srivastava and Ansari, 1988; Viana et al., 1996; Cameron et al., 1993), despite no improvement of the hyperglycemia. Based on the above results one can conclude that patients with type 1 and type 2 diabetes may benefit from a higher intake of vitamin E than the current recommendation.

The greater stability of vegetable oils versus animal fats under oxidative conditions is known to be due to the higher levels of natural antioxidants in the oils. An important and commonly occurring class of natural antioxidants in vegetable oils is tocopherols (T) of vitamin E family. There are 8 naturally occurring forms of vitamin E; α-, β-, γ-, δ-tocopherols and tocotrienols (T3). Tocotrienols are minor plant constituents especially abundant in cereal grains (such as barley, oat, wheat and rye), rice bran, palm oil and latex (Kasparek, 1980b). The vitamin E antioxidant property reflects the similarity in chemical structures of T and T3, which differ only in possessing a farnesyl or unsaturated phytol side chain, respectively (Kasparek, 1980a) (Fig. 1.8). Tocopherols predominate in certain oils such as corn oil, soybean oil and olive oil. Whereas, the T3 series predominates in rice bran oil (RBO), palm oil and barley oil. Small amounts of T3 are found in carrots, sweetcorn and germ oils (Shin and Godber, 1994). Several lines of research have established that populations, which consume large amounts of cereal grain and vegetable oils, tend to have a lower incidence of cardiovascular disease (Sacks et al., 1975; Burstem et al., 1978; Gould et al., 1980). Furthermore, studies on cereal grains demonstrated that barley is particularly effective in lowering lipid levels in animal models (Qureshi et al., 1980a; Qureshi et al., 1980b; Qureshi et al., 1980c). The ability of barley extracts to lower
<table>
<thead>
<tr>
<th>Position of methyl group</th>
<th>Tocotrienols</th>
<th>Tocopherols</th>
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<tr>
<td>5,7,8-Trimethyl</td>
<td>(\alpha-T_3)</td>
<td>(\alpha-T)</td>
</tr>
<tr>
<td>5,8-Dimethyl</td>
<td>(\beta-T_3)</td>
<td>(\beta-T)</td>
</tr>
<tr>
<td>7,8-Dimethyl</td>
<td>(\gamma-T_3)</td>
<td>(\gamma-T)</td>
</tr>
<tr>
<td>8-Monomethyl</td>
<td>(\delta-T_3)</td>
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**Lovastatin**

![Molecular structure of Lovastatin](image)

*Fig. 1.8. Molecular structures of tocotrienol, tocopherol isomers and Lovastatin.*
l lipids in vivo led to the purification and identification of biologically active compound tocotrienols (Qureshi et al., 1986). There are scattered reports that neither rice bran nor RBO lowered cholesterol levels. These findings may be explained by reports that some, but not all, rice cultivars contain tocotrienols, which exert a powerful hypocholesterolemic action (Qureshi et al., 1986; Qureshi et al., 1989). Qureshi et al. (1986) have demonstrated the hypocholesterolemic effect of tocotrienols isolated from barley, oats, rice bran and palm oil in various animal models. Anticholesterol impact of tocotrienols has also been demonstrated in hypercholesterolemic subjects (Qureshi et al., 1995). Out of α-, β-, γ- and δ-T3, γ- and δ-T3 have been found to be most potent in terms of their HMG-CoA reductase inhibition as well as cholesterol lowering effects. The efficiency of hypocholesterolemic action as well as the degree of inhibition of HMG-CoA reductase activity mediated by α-T3 was substantially lower than γ- and δ-T3 (Pearce et al., 1992). β-form of T3 failed to exhibit any anticholesterol activity. Rice bran oil is the richest source of T3, whereas corn, groundnut, mustard, soybean and coconut oils and butter fat contain only T, which have no lipid lowering effect. The T3 are highly effective in lowering total blood cholesterol and LDL-cholesterol apparently by reducing the HMG-CoA reductase activity. The T on the other hand does not inhibit cholesterol synthesis and thus do not lower serum cholesterol. A dose dependent effect of tocotrienol rich fraction (TRF) isolated from palm oil was observed for lowering the serum cholesterol and LDL-cholesterol in normolipidemic and hypercholesterolemic swine, quail and chicken (Pearce et al., 1992; Qureshi and Qureshi, 1993).

In several respects T3 appear to operate in similar manner to oxysterols. Certain oxysterols have been shown to regulate cholesterol biosynthesis by transcriptional down-regulation of reductase gene (Kandutsch et al., 1978; Schroepfer et al., 1979; Schroepfer et al., 1981; Schroepfer et al., 1982). It has been postulated that endogenously produced oxysterols are natural regulators of cholesterol biosynthesis. These oxysterols are potent repressors of HMG-CoA reductase and bind strongly to cytosolic oxysterol binding protein (Spencer et al., 1985; Saucier et al., 1985). Since oxysterols are natural regulators of cholesterol biosynthesis and act by suppressing HMG-CoA reductase gene, the T3 may have a similar function, but it acts
at post-transcriptional level as has been experimentally demonstrated in HepG2 cells (Parker et al., 1993). The human hepatoma HepG2 cell culture model was employed to compare the intrinsic activities of T3. In HepG2 cells, inhibition of sterol synthesis correlates with rapid suppression of HMG-CoA reductase when incubated with T3. The recemic synthetic tocotrienols exhibit comparable biological activity to the natural tocotrienols in the cholesterol suppression activity. Gamma-Tocotrienol has been shown to mediate the suppression of enzymatic activity and protein mass of HMG-CoA reductase in HepG2 cells, through decreased synthesis (57% of control) and enhanced degradation (2.4 fold versus control) of the enzyme. This increased degradation was regulated through the increased cellular conversion of farnesyl to farnesol, which then signal the enhanced proteolytic degradation of HMG-CoA reductase. Thus, tocotrienols influence the mevalonate pathway in mammalian cells in vitro, by post-transcriptional suppression of HMG-CoA reductase, and appear to specifically modulate the intracellular mechanism for controlled degradation of the reductase protein (Parker et al., 1993). In addition, another report indicates that γ-tocotrienol influences apoB secretion by both cotranslational and posttranslational processes involving a decreased rate of apoB translocation and accelerated degradation of apoB in HepG2 cells. This activity correlated with a decrease in free and esterified cholesterol (Theriault et al., 1999a). Without or lower level of apo-B, VLDL, the precursor of LDL, is unable to assemble with core lipids and be secreted from the liver. Thus, the ability of tocotrienol to reduce apo-B plasma levels depends largely on both the clearance rate of LDL and the production rate of VLDL (Theriault et al., 1999b). Taken together, the information indicates an association between the suppression of hepatic cholesterol synthesis and apoB secretion, and the observed lowering of apoB and LDL-C levels in animal and human models (Theriault et al., 1999b). However, elucidation of precise in vivo mechanisms of TRF-mediated inhibition of HMG-CoA reductase at molecular level remains to be investigated. It was observed that TRF preparations containing 25 % or more α-tocopherol could attenuate the cholesterol-suppressive action of γ-tocotrienol by increasing HMG-CoA reductase activity but without affecting serum cholesterol level (Qureshi et al., 1995; Qureshi et al., 1996). Therefore, it appears that the attenuation observed in vivo might be related to a preferential transport of α-tocopherol in serum lipoproteins (Qureshi et al., 1996). Our laboratory has previously reported that feeding of TRF or purified
TRF, isolated from refined edible grade RBO, to normal rats for two weeks was associated with a significant decline in plasma TG, TC, LDL-C, including apoB levels. HDL$_3$-C, which is considered as strong predictor of the presence and extent of CAD was significantly increased in TRF, treated normolipidemic rats. TRF feeding to rats along with an atherogenic diet for three weeks significantly prevented the rise in plasma TG, TC, LDL-C, apoB, HDL-C, apoA-1 and HDL$_3$-C levels in comparison to rats fed atherogenic diet alone. Five and seven days after the withdrawal of atherogenic diet, plasma and lipoprotein lipid levels including apoB and apoA-1 were reduced. Treatment of hyperlipidemic rats with purified TRF resulted in a further significant reduction in the above parameters indicating the efficacy of TRF in the treatment of experimental hyperlipidemia. The minimum dose of TRF or purified TRF required to exert the maximum hypolipidemic effect in normolipidemic and hyperlipidemic rats has been found to be 8 mg TRF or 5.2 mg purified TRF/day/Kg body weight. It has also been demonstrated that cholesterol lowering property of tocotrienols in normolipidemic and hyperlipidemic rats is due to suppression of enzymatic activity and protein mass of HMG-CoA reductase (Minhajuddin et al., 1999; Minhajuddin et al., 2005). Administration of TRF enriched with tocotrienols and tocopherols, resulted in a significant decline in microsomal lipid peroxidation (TBARS) and plasma LDL oxidation (conjugated dienes) in normolipidemic as well as in response to oxidative stress, evoked in experimental hyperlipidemia in rats (Minhajuddin et al., 1999; Minhajuddin et al., 2005). These results also demonstrate a differential hypolipidemic impact of purified TRF isolated from four cultivars of rice, raw Basmati, Saket-4, Sarju-52, and Mansuri, due to the difference in their $\gamma$- and $\delta$-T3 content, in hyperlipidemic rats. Based on total content of $\gamma$- and $\delta$ -T3 present in the purified TRF of each cultivar, hypolipidemic efficacies at an equivalent dose of 3 and 6 mg TRF/day/Kg body weight (calculated on the basis of combined content of $\gamma$- and $\delta$ -T3 present in 3 and 6 mg of TRF) caused a dose-dependent decline in plasma and lipoprotein lipids including apoB, HMG-CoA reductase activity and it’s protein mass, formation of TBARS and conjugated dienes of plasma LDL (Beg et al., 2000a; Beg et al., 2000b). Our laboratory has also demonstrated a long-term therapy of a FH patient with severe xanthomas. Treatment at a dose of 8 mg TRF/day/Kg body weight for 20 weeks caused a significant reduction in plasma TG, TC, LDL-C, and apoB levels with a substantial increase in the levels of HDL-C, HDL$_2$-C and apoA-1. TRF
also caused a substantial improvement in the ratios of LDL-C/HDL-C, apoB/apoA-1 and HDL-C/TC, indicating the normalization of lipid parameters. Consistent with reduction in lipid parameters, after TRF treatment rapid growth of skin xanthomas was arrested. In addition, a significant regression of xanthomas on buttocks extending to thighs, elbows and knee was observed (Beg et al., 1997).

Based on strong hypocholesterolemic and antioxidant properties of tocotrienols, our laboratory has also investigated antitumour activity of tocotrienols in experimental carcinogenesis of mammary gland and liver. The carcinogen, 7, 12-dimethylbenz (α) anthracene (DMBA), which is known to induce both mammary carcinogenesis and hypercholesterolemia in rats, was utilized. As expected, six months after administration of DMBA, a significant increase in plasma TG, TC, LDL-C, including apoB, HMG-CoA reductase activity, microsomal lipid peroxides (TBARS), conjugated dienes of LDL oxidation, plasma, liver and mammary gland alkaline phosphatase and glutathione-S-transferase levels. DMBA treatment also resulted in the formation of neoplastic nodules as multiple tumors on mammary glands and greyish white patches on the livers of rats. Feeding of TRF to rats, during pre-and post-initiation stages, was associated with a significant decline in the above parameters. In addition, examination of gross morphology and histology suggested that dietary TRF did offer a significant protection and did reduce the severity and extent of neoplastic transformation during both initiation and/or promotion in both mammary glands and livers of carcinogenic rats. TRF treatment, in addition to its anticancer and antioxidant impacts also exerted a strong hypocholesterolemic action, indicating a linkage between atherosclerosis and cancer. The dual chemopreventive actions of TRF in atherosclerosis and cancer are apparently mediated by reducing HMG-CoA reductase, thus limiting the availability of mevalonate derived products required for cholesterol production and tumor growth (Iqbal et al., 2003).

Our laboratory has also reported a strong hypolipidemic action of TRF, when administered to type 2 diabetic patients with hyperlipidemia. In particular TC and LDL-C, which are positively associated with CHD, were significantly reduced to normal levels (Baliarsingh et al., 2005). However, hypoglycemic effect of TRF was not observed in these patients because they were glycemically stable and their glucose and HbA₁ levels were close to normal values. Recently, our laboratory has also
demonstrated that feeding of 16.2 mg % TRF (isolated from rice bran oil) or 6.97 mg-% Tocomin together with a cholesterol-rich diet to rabbits for 22.4 weeks significantly prevented the increase in Plasma TG, TC, VLDL-C, and LDL-C levels with no effect on elevated plasma HDL-C, HDL3-C and HDL2-C levels. In response to oxidative stress evoked in experimental hyperlipidemia in rabbits, as reflected by increased formation of plasma lipid peroxides, higher base line levels of diene conjugation of LDL, as modified in vivo, increased rate of conjugated diene formation in LDL and decrease in lag phase time of LDL oxidation in vitro, was substantially blocked by TRF or Tocomin when fed together with cholesterol-rich diet. Consistent with above results, feeding of the cholesterol rich diet was also associated with the formation of fatty streak lesions in the aortas of hyperlipidemic rabbits, which was significantly reduced in TRF or Tocomin supplemented groups. We have also demonstrated that after 10 weeks of feeding to rabbits either a 0.33 % cholesterol-rich diet or the same diet containing 0.33 % cholesterol of which 5 % was oxidized, plasma TG, TC including FC and EC, VLDL-C, LDL-C, HDL-C and its subfractions, HDL2-C and HDL3-C were substantially increased but to a similar extent supplementation of 50 mg % TRF to these diets significantly blocked the increase in the above lipid parameters except HDL-C, HDL2-C and HDL3-C levels were not reduced significantly. The oxidative stress, evoked in oxidized cholesterol-fed rabbits was significantly higher than nonoxidized cholesterol-fed rabbits. This differential effect was reflected in several indices of oxidative stress, such as plasma and liver lipid peroxides, base line levels of ex vivo diene conjugation of LDL, rates of conjugated diene formation and TBARS of LDL, and lag phase time of in vitro LDL oxidation. These oxidative parameters were substantially more pronounced in rabbits fed oxidized cholesterol, apparently due to the presence of cholesterol oxidation products. Tocotrienols being very potent antioxidants, significantly blocked the above mentioned oxidative parameters in both groups. However, tocotrienols were significantly more potent in blocking the above parameters when supplemented with a diet enriched in oxidized cholesterol. Consistent with the above results, feeding of oxidized cholesterol-rich diet resulted in the formation of fatty streak lesions in the aorta of rabbits, which was 2-fold higher than rabbits fed a nonoxidized cholesterol-rich diet. Consistent with a strong hypolipidemic and antioxidant action of tocotrienol, supplementation of TRF with oxidized cholesterol blocked the formation of fatty
streak lesions by 4.1-fold, whereas, TRF feeding together with nonoxidized cholesterol caused a reduction of 2.4-fold only (Zainuddin, 2004: Beg and Zainudin, 2003). In another report, Teoh et al. (1994) have demonstrated that feeding of an atherogenic diet containing tocotrienols to rabbits for 12 weeks significantly reduced serum lipid peroxides and mediated a significant protection against atheroma in rabbit aorta. Nazaimoon and Khalid (2002) have reported that feeding of TRF-rich diet effectively prevented increase in AGE in normal rats, and caused decrease in blood glucose and HbA1 in diabetic rats. Despite the fact that TRF-rich diet mediated a significant decrease in blood glucose and HbA1 levels, the values measured at week-8 and week-12 were still within the diabetic range.

During the past few years, two novel tocotrienols were isolated from stabilized and heated rice bran, apart from the known α-, β-, γ-, and δ-, tocopherols and tocotrienols. These new tocotrienols were separated by HPLC, using a normal phase silica column. Their structures were determined by ultraviolet, infrared, nuclear magnetic resonance, circular dichroism, and high resolution mass spectrosocopies and established as desmethyl tocotrienol and didesmethyl tocotrienol. These tocotrienols significantly lowered serum total and LDL cholesterol levels and inhibited HMG-CoA reductase activity in chickens. They had much greater in vitro antioxidant activities and greater suppression of B16 melanoma cell proliferation than α-tocopherol and known tocotrienols. These results indicated that the number and position of methyl substituents in tocotrienols affect their hypocholesterolemic, antioxidant and antitumour properties (Qureshi et al., 2000). Feeding of these two novel tocotrienols to hereditary hypercholesterolemic swines for 6 weeks caused a significant reduction in TG, TC, LDL-C, apo-B, platelet factor 4, thromboxane B2 and hepatic HMG-CoA reductase activity (Qureshi et al., 2001a). These results are consistent with the hypocholesterolemic effects of these two novel tocotrienols in chickens (Qureshi et al., 2000). The reduction in cholesterol level may be due to inhibition of cholesterol biosynthesis at the level of HMG-CoA reductase through a post-transcriptional mechanism involving protein degradation as shown earlier for other tocotrienols (Parker et al., 1993). Desmethyl tocotrienol and didesmethyl tocotrienol also mediated a significant decrease in serum TG, TC, LDL-C, apoB, Lp(a), platelet factor 4 and thromboxane B2 levels of hypercholesterolemic humans after a double blind,
12-week study (Qureshi et al., 1997). Steiner et al. (1995) reported a greater reduction in platelet adhesiveness with a concomitant reduction in ischemic events in the patient group who were taking α-tocopherol plus aspirin compared to those taking aspirin alone. In several epidemiological studies, tocotrienols, both as a TRF and purified γ-tocotrienol, was shown to reduce the synthesis of an eicosanoid, namely thromboxane B2. The mechanism(s) involved for tocotrienols-mediated antithrombotic effect remains uncertain, but may be similar to that reported for α-tocopherol (Theriault et al., 1999b). In addition, δ-tocotrienol improves vascular functions, reducing adhesion molecules over that of α-tocopherol (Chao et al., 2002). These studies support the concept that tocotrienols mediate a potentially important and novel mechanism(s) in controlling atherogenesis. It is interesting to note that these two newly discovered tocotrienols are more effective in terms of cholesterol lowering activity than α-, γ- and δ-tocotrienols (Qureshi et al., 2000; Qureshi et al., 2001a). Recently, a dose-dependent effect of tocotrienol rich fraction containing mixture of the novel desmethyl- and didesmethyl tocotrienols (TRF25) has been investigated in hypercholesterolemic humans (Qureshi et al., 2002). The results showed that intake of a dose of 100 mg/day of TRF25 for 35 days caused maximum decrease in serum TC, LDL-C, apoB and TG levels when compared to baseline values (Qureshi et al., 2002). Administration of a 4-week dietary supplement of either 58 mg γ-T3 or mixture of tocotrienols as TRF (29.5 % α-T3, 3.3 % β-T3, 41.4 % γ-T3 and 0.1 % δ-T3) per day per Kg body weight to hamsters receiving a high fat diet revealed that γ-T3 was more potent hypocholesterolemic agent than TRF (Raederstorff et al., 2002). The synergistic effect of TRF25 has been reported in hypercholesterolemic humans. Administration of TRF in combination with lovastatin to hypercholesterolemic humans for 35 days exerted a synergistic lipid lowering effect, when compared to values obtained from subjects given TRF25 or lovastatin alone (Qureshi et al., 2001b). The other beneficial effects of tocotrienols include inhibition of angiogenesis, which is important in tumor growth, diabetic retinopathy, rheumatic arthritis, wet-type macular degeneration via vascular endothelial growth factor (VEGF) inhibition (Miyazawa, et al. 2004). Delta-tocotrienol and secondarily γ-tocotrienol are reported to be involved in the correction of genetic defects in nerve protein synthesis in children with familial dysautonomia, a neurodegenerative genetic disorder (Anderson et al., 2003). Tocotrienols at concentrations well below their antioxidative properties.
(4-10 times lower) are effective in preventing glutamate-induced neuronal cell deaths (Sen, et al., 2000; Khanna et al., 2003). Other unique beneficial effects of tocotrienols, especially the desmethyl isomers, not shared by α-tocopherol include natriuresis, antihypertension, anti-inflammatory and antiosteoporesis (Tan, and Llobrera, 2005). An encouraging development in the treatment of hyperlipidemia has been the introduction of a new class of fungal-derived compounds (statins) that are potent competitive inhibitors of HMG-CoA reductase, the rate controlling enzyme in the biosynthetic pathway for cholesterol. Statins have been described as the most potent class of drugs to reduce serum cholesterol levels and coronary morbidity and mortality in patients with or without CAD (Maron et al., 2000; Vaughan et al., 2000).

It is also effective in patients with hypercholesterolemia associated with diabetes mellitus or with the nephrotic syndrome. The main effect of statins on the lipid profile is therefore to lower LDL-C levels, which they do by increasing efficacy on a per mg basis from fluvastatin to pravastatin to simvastatin and lovastatin to atorvastatin (Jones et al., 1998). These agents also reduce triglycerides by upregulating the hepatic LDL receptor and probably by reducing VLDL production. Initially, statins showed to be beneficial in patients with substantially elevated cholesterol (The Scandinavian Simvastatin Survival Study (4S), 1994; Shepherd, 1995), their benefits have currently been extended to use in patients with average cholesterol levels (LIPID Study Group, 1998; Downs et al., 1998). Although there is no association between blood cholesterol levels and the incidence of stroke (PSC, 1995), statins reduce the risk of stroke in patients with CAD (Vaughan et al., 2001; Schwartz et al., 2001). In addition, recent studies showed that statin therapy over a six month period markedly inhibits the inflammatory components of multiple sclerosis (Vollmer et al. 2004) and that statin therapy is associated with a decrease rate of severe sepsis (Almog et al. 2004), two pathological conditions thought to be independent of cholesterol levels. All these observation lead to the speculation that statins might influence vascular biology through non-lipid mechanisms (Vaughan et al., 1996; Bellosta et al., 2000). Being competitive inhibitors of HMG-CoA reductase, statins, block synthesis of cholesterol in the liver, thereby triggering compensatory reactions that lead to a reduction in plasma LDL. Consequently, statins also prevent the catabolism of important isoprenoid intermediates of the cholesterol biosynthetic pathway such as farnesyl pyrophosphate and geranyl geranyl pyrophosphate (Goldstein and Brown, 1990), and
thus exert effects completely independent of cholesterol synthesis. These isoprenoid intermediates serve as important lipid attachments for the post-translational modification (prenylation) of a large variety of proteins, including small GTP-binding proteins Ras and Ras-like proteins (Rho, Rab, Rac, Ral, and Rap) (Van Aelst and D’Souza-Schorey, 1997). Prenylation of these proteins are necessary for a large number of physiological functions such as cell shape, cell mobility and proliferation (Casey, 1995; Laufs and Liao, 2000; Takai et al., 2001). In endothelial cells, Ras activity is dependent on farnesylation, whereas Rho is dependent on geranylgeranylation (Van Aelst and D’Souza-Schorey, 1997). By inhibiting HMG-CoA reductase, statins, prevent Ras and Rho isoprenylation, leading to the accumulation of inactive proteins in the cytoplasm. Increasing evidence suggest that statins exert some effects that are totally independent of LDL-C reduction. Pleiotropic effects of statins include beneficial effects on endothelial function, as well as anti-inflammatory and immunomodulatory properties, most of these effects seem to be mediated by the inhibitory effect of statins on isoprenoid intermediates, and in particular by the inhibition of Rho signaling pathway. Finally, statins may also improve endothelial function through their antioxidant effects. It has been shown that statins inhibit the production of ROS, such as superoxide or hydroxyl radicals (Rikitake et al., 2001). Recently, Haendeler et al. (2004) have demonstrated that statins mediate S-nitrosylation of thioredoxin, which enhances the enzyme activity, resulting in a significant reduction in intracellular ROS in endothelial cells. Statins also attenuate Ang II-induced ROS production by inhibiting Rac-1-mediated NADH oxidase activity (Wassmann et al., 2001). Wassmann et al. (2002) have demonstrated that atorvastatin exerts cellular antioxidant effects in cultured rat vascular smooth muscle cells and in the vasculature of spontaneously hypertensive rats mediated by decreased expression of essential NAD(P)H oxidase subunits and by upregulation of catalase expression.

As discussed above increased oxidative stress in diabetes mellitus results in increased lipid peroxidation. Oxidized lipoproteins, particularly LDL and other lipid peroxidation products may accelerate atherosclerotic process. Therefore, the favorable effect of statins in preventing or reducing atherosclerosis may be partially due to a reduction of lipid peroxidation. Indeed, some reports suggest that statins have an
antioxidant potential (Kleinveld et al., 1993; Human et al., 1997). Singh et al. (1997) have demonstrated that feeding of a trans-fatty acids-rich diet supplemented with lovastatin to rabbits for 24 weeks was associated with a low but significant decrease in lipid peroxides and conjugated dienes, indicating that lovastatin may have antioxidant activity. In another study, Chen et al. (1997), have reported that feeding of a cholesterol-rich diet to rabbits supplemented with lovastatin for 14 weeks caused a substantial and significant decline in serum MDA levels. In addition, lovastatin mediated a restoration in the plasma SOD activity, which was significantly decreased in rabbits fed only cholesterol. In both the studies the fatty streak lesion areas were significantly reduced in lovastatin treated rabbits. In a combined model of STZ-induced diabetes and high saturated fat and cholesterol fed hypercholesterolemia in Syrian hamsters, lovastatin supplementation may contribute to the prevention of foam cell formation by inhibiting the oxidation of lipoproteins. Lovastatin decreased plasma lipid peroxide level, increased the lag time, and reduced conjugated diene formation during \textit{in vitro} LDL oxidation (El-Swefy et al., 2000). Yamaguchi et al. (2004) have demonstrated that aqueous extract of cigarette smoke facilitates oxidative modification of LDL via peroxynitrite \textit{in vitro} and in hypercholesterolemic WHHL rabbits, and that this oxidation and nitration of LDL can be effectively prevented by treatment with fluvastatin. Fluvastatin has an efficient peroxynitrite scavenging ability, which may be potentially beneficial to hypercholesterolemic patient with oxidative stress, such as smoking and inflammation. In a recent study (Manuel-y-Keenoy et al., 2004), treatment of type 1 diabetic patients with 20 mg/day atorvastatin revealed a significant decline in plasma MDA levels after 3- and 6 months of treatment. However, total TBARS production, which is a measure of \textit{in vitro} lipid peroxidation, was significantly increased after 3- and 6 months of treatment.

The efficacy of statins for prevention of cardiovascular events has been established in a number of landmark randomized clinical trials (Shepherd et al. 1995; HPSCG, 2002; Sever et al. 2003), however, whether statin has beneficial effects on glucose metabolism still remains controversial. The West of Scotland Coronary Prevention Study (WOSCOPS) demonstrated a significant risk reduction in the development of type 2 diabetes in hypercholesterolemic patients treated with pravastatin (Freeman et al., 2001), while several studies with other statins have not
shown this beneficial effect on glucose metabolism (HPSCG, 2002; Sever et al., 2003). In addition, it has recently been reported that intake of high concentrations of atorvastatin was significantly associated with worsening of glycemic control after acute coronary syndrome (Sabatine et al., 2004), suggesting the protective effect unique to pravastatin. Many CAD patients with abnormal lipid metabolism also have impaired glucose metabolism; together these are key component of metabolic syndrome. Postprandial metabolic disorder including hyperglycemia and hyperinsulinemia, are involved in the process of atherogenesis, which can lead to major clinical cardiovascular events. Postprandial hyperglycemia is defined as impaired glucose tolerance (IGT), following an oral glucose tolerance test (OGTT). IGT has been shown to be a significant risk factor for CVD, and is also considered to presage the onset of type 2 diabetes mellitus, which is associated with a significantly worse prognosis in patients with CAD (DECODE Study Group, 1998). Now, it is recognized that IGT may be a practical treatment target to prevent the development of overt diabetes and a modifiable risk factor for CVD (ADANIDDKD, 2002).

Adiponectin is an adipocyte-derived secreted protein that has several important metabolic and endocrinologic functions, which are of particular relevance to glucose metabolism and the development of type 2 diabetes (Matsuzawa et al., 2004). Patients with CAD have decreased adiponectin levels (Hotta et al., 2000) and hypoadiponectinemia is associated with an increased risk of MI (Pischon et al., 2004). In humans, increased serum concentrations of adiponectin are associated with increased insulin sensitivity and glucose tolerance (Goldfine, and Kahn, 2003). Sugiyama et al. (2006) have recently reported that pravastatin treatment of type 2 diabetics with IGT and CAD was associated with a significant improvement in glucose tolerance and significant increase in plasma levels of adiponectin. Therefore, pravastatin therapy may be an advantageous clinical option to improve hyperlipidemia and glucose metabolism in patients with CAD.
1.7 Scope of the Present Study

As discussed above, hyperglycemia is the most important factor in the onset and progress of diabetic complications mainly by producing oxidative stress (Giugliano et al., 1996). Altered cellular metabolism caused by hyperglycemia has been suggested to play an important role in increasing the risk of cardiovascular, renal, ophthalmic and neurological complications of diabetes mellitus (Brownlee et al., 1984; Brownlee et al., 1988; Nathan, 1993). The hyperlipidemia and hyperketonemia associated with diabetes may also lead to increased lipid peroxidation. The excessive nonenzymatic glycosylation of proteins including LDL, associated with substantially increased superoxide, hydrogen peroxide, and hydroxyl radical production, stimulate formation of advanced glycosylation end products and foam cells, which cause extensive cellular and tissue damage, including vascular injury (Lyons, 1991; Epstein, 1989). Since most animal studies of diabetes are induced by administration of STZ or alloxan, toxic agents that target β-cells, they are thus essentially considered as models of type 1 diabetes. Nevertheless, such animals show increased lipid peroxidation, hyperlipidemia and other diabetic complications seen in type 2 diabetes (Halliwell, 2002). In addition, hyperglycemia causes cellular dysfunctions (Koya and King, 1998; Nishikawa et al., 2000), glycation (Brownlee, 1994) and oxidative stress (Wolf, 1993). All these factors aggravate the consequences of the diabetic dyslipidemia by increasing susceptibility to lipid peroxidation. This leads to accumulation of products of oxidative damage to lipids (Griesmacher et al., 1995), foam cell formation and eventually increased atheroma plaque deposition (Ross, 1990). In normal subjects, oxidative stress is reduced or prevented by the “free radical scavengers” present in plasma. Enzymes such as SOD, catalase, Gpx and nonenzymatic scavengers, such as GSH, vitamins A, C, and E are known to readily react with free radicals and minimize the damage. Thus, the overall antioxidant status in plasma including erythrocytes and other tissues seems to have an important role in the etiology of diabetic complications. Increased lipid peroxidation can be detected in early stages of type 2 diabetes, well before the development of diabetic complications (Halliwell, 2002). Since lipid peroxides are well known to cause tissue damage, it is likely that nephropathy, retinopathy, endothelial dysfunction and peripheral neuropathy associated with poor diabetic control involve free radical damage (Halliwell and Gutteridge, 1984; Oranje and Wolffenbuttel, 1999; Devraj and Jialal,
2000; Bursell et al., 1999; Low et al., 1997). Published reports (Halliwell, 2002) indicate beneficial effects of antioxidants such as vitamin E (α-T) on enhanced oxidative stress associated with diabetes, when 600-1200 units of α-T/day were given to diabetic patients for 2-6 months. The protective impacts of α-T include decrease in lipid peroxidation, free radical production, C-reactive protein; improved retinal blood flow and renal dysfunction and improved nerve function with no effect on protein glycation and HbA1 levels. In contrast, Skrha et al. (1999, 1997) found that intake of 600 mg of α-T by type 2 diabetics for 3 months appeared to worsen diabetic control and raised HbA1 levels. The effectiveness of α-T supplementation in preventing glomerular dysfunctions (Koya and King, 1998) and normalizing the abnormal retinal blood flow (Kunisaki et al., 1998) has also been reported in diabetic rats, where α-T has been shown to act directly on the diacylglycerol-protein kinase-C pathway. Vitamin E could also exert it’s protective effects indirectly by reducing free radical mediated damage to islet of β-cells and thus improving insulin action (Slonim et al., 1983; Paolisso et al., 1993; Tanaka et al., 1982). However, published reports indicate that vitamin E treatment during diabetes failed to reduce blood glucose levels in hyperlipidemic-diabetic hamsters (El-Swefy et al., 2000) and in diabetic rats (Nazaimoon and Khalid, 2002). Similarly, in diabetics, vitamin E intake was not associated with any decline in blood glucose levels (Cerriello et al., 1991).

Improved glycemic control has been shown to retard the progression of diabetic complications particularly microvascular disease (UKPDS, 1998; Cusick et al., 2002). Different types of oral hypoglycemic agents, such as begunoids and sulphonylureas are available along with insulin for the treatment of diabetes mellitus (Holman and Turner, 1991). However, these drugs commonly used in the treatment of hyperglycemia in diabetics, but they are unable to lower glucose concentration to normal levels. In addition, these drugs are unable to restore normal pattern of glucose homeostasis on permanent basis. Furthermore, use of these hypoglycemic agents is restricted by their pharmacokinetic properties, secondary failure rates and host of side effects (Kameshwara et al., 1997; Valiathan, 1998; Melinda, 1988). Imperfect normalization of glucose metabolism by replacement insulin therapy may alter the concentrations and compositions of potentially atherogenic lipoproteins (Bierman and Glomset, 1992).
Thus, there is a need to seek newer and alternative approaches for effective therapy in the management of hyperglycemia.

Although DCCT indicated that blood glucose is highly predictive of microvascular disease (DCCTRG, 1993), the contribution of all the commonly measured risk factors can explain no more than 25% of the excess macrovascular CAD associated with diabetes (Pyorala et al., 1987). The dyslipidemic profile of diabetics includes increased levels of TG, TC, VLDL-C and LDL-C, increased sd-LDL, glycation of LDL and decreased plasma HDL concentration (Grundy et al., 1999). Recent reviews indicate that altered plasma lipoprotein profile in the excess atherosclerosis associated with diabetes may be most critical, because at any TC level, diabetics have 3-to 5-fold higher CAD mortality rates than do nondiabetic subjects (Steiner, 1994). Consistent with these findings, in type 2 diabetics, the total plasma antioxidant capacity, susceptibility of LDL to oxidation and excretion of oxidized products of arachidonic acid are all increased in proportion to the severity of hyperglycemia (Grundy et al., 1999; Ceriello et al., 1997; Peuchant et al., 1997). The involvement of increased oxidative stress in diabetes is supported by several data such as increased concentrations of lipid peroxidation products (conjugated dienes, hydroperoxides and TBARS) and decreased levels of antioxidants in the plasma of patients (Sato et al., 1979; Karpen et al., 1984; Wolf, 1987). In addition, an elevated glucose concentration in plasma could promote LDL oxidation by leading to glycated forms of the particle, which are more sensitive to transition metal-dependent autooxidation (Sakurai et al., 1991). High glucose concentrations have been reported to enhance in vitro LDL oxidative modification induced by copper ions (Hunt et al., 1990; Hunt et al., 1994). A number of reports described shortened oxidative lag phase during copper-catalyzed oxidation of LDL and glycated LDL from diabetic patients. Increased TBARS were found in LDL and erythrocyte membranes of type 1 and type 2 diabetic patients after exposure to phenylhydrazine (Rabini et al., 1994). Vitamin E supplementation of glycated LDL during in vitro oxidation decreases its susceptibility to oxidation (Li et al., 1996). Oxidative reactions also can contribute to enhanced glycation. However, a number of studies failed to show an effect of α-T on LDL glycation (Reaven, 1995; Li et al., 1996; Rabini et al., 1994). Elevated glucose concentrations in the culture medium enhances superoxide production and the
subsequent ability of endothelial and smooth muscle cells to oxidatively modify LDL (Mazeri et al., 1995). Otero et al. (2002) have demonstrated that the consumption of vitamin E in normal human LDL subjected to Cu^{++}-induced oxidation was delayed by a glucose concentration frequently found in subjects with poorly controlled type 1 diabetes. However, high glucose concentration accelerated the LDL oxidation once LDL associated vitamin E was consumed. This prooxidant effect of glucose was reflected by increased formation of conjugated dienes, TBARS and a decreased lag phase (Otero et al., 2002). Although lipid oxidation in the vessel wall is thought to occur as result of local deficiency of endogenous antioxidants or an excess of free metal ions, only limited data support these hypotheses (Jialal and Devraj, 1996; Steinberg, 1997). Research has shown that human atherosclerotic plaques contain massive amounts of lipid peroxidation products, despite the presence of large quantities of α-tocopherol and ascorbate (Suarna et al., 1993). Thus, it is unclear whether oxidized lipoproteins originate in the arterial wall or are produced in the circulation and then enter the intimal space. In another study, vitamin E decreased LDL susceptibility to oxidation and reduced the plasma level of lipid peroxides with no change in plasma lipoprotein profiles in hyperlipidemic-diabetic hamsters. In addition, vitamin E, despite its effect on reducing oxidative stress markers and lowering plasma TG levels, was not effective in inhibiting foam cell formation in the aortic arch of hyperlipidemic-diabetic hamster model (El-Swefy et al., 2000). However, hypolipidemic drug coupled with antioxidant properties were more effective in reducing clinical manifestations of long-term diabetes in experimental animals (Cameron et al., 1993). Similarly, several intervention trials (GISSI-Prevenzione Investigators, 2001; Keith et al., 2001) with high doses of vitamin E for prolonged periods failed to positively affect cardiovascular events in diabetics with CHD. Nevertheless, oxidative modification of LDL is one possible mechanism for an early development of atherosclerosis in diabetes. It is possible that increased atherogenicity of LDL during diabetes is associated with a preponderance of sd-LDL subpopulation, that is more prone to oxidative modification than lb-LDL (Chancharme et al., 1999). Several studies have reported a 2- to 3-fold increase in CHD risk among patients with a predominance of sd-LDL particles (Austin et al., 1988; Austin et al., 1990; Austin et al., 1994). Recently, Koba et al. (2006) have reported that prognosis of CHD was closely linked not to the LDL particle size but to the concentration of sd-LDL. Therefore, sd-LDL has been highlighted as a new and
useful marker for the risk of CHD or type 2 diabetes with and without CHD. In addition, because of greater preponderance of sd-LDL, a moderately high LDL-C (between 130 and 160 mg/dl) in a type 2 diabetic patient is equivalent to a much higher LDL-C in terms of CHD risk for a nondiabetic subject (Grundy et al., 1999; Haffner et al., 1998; NCEP Expert Panel, 1993). Therefore, the primary target of therapy in type 2 diabetic patients is lowering LDL-C to a level ≤ 100 mg/dl as suggested by NCEP Expert Panel for patients with preexisting CHD (Haffner et al., 1998). Similarly, the substantial increase in more atherogenic sd-LDL in diabetic patients with and without CHD requires an immediate attention. At present, the most common LDL-C lowering agents are statins. However, they have been reported to exhibit host of side effects (Haffner et al., 1998; Assmann et al., 1998). To the best of our knowledge, no therapeutic interventions to specifically reduce the elevated levels of highly atherogenic sd-LDL in diabetic-hyperlipidemic patients or animals have been reported (Hirano et al., 2004; Hirano et al., 2005; Koba et al., 2006).

Several alterations in structural and dynamic properties of erythrocytes membrane have been reported in type 1 and type 2 diabetes (Watala, 1993). Of particular interest has been the observation of an altered activity of erythrocytes membrane Na⁺, K⁺-ATPase in type 1 and type 2 diabetes (Rizvi and Zaid, 1998; Kiziltunc et al., 1997). Impaired Na⁺, K⁺-ATPase is a feature of diabetes in many cell types and is believed to be a pivotal regulator of various cell functions (Mahesh and Menon, 2004). Increased erythrocytes MDA concentrations are known to cause a decrease in the fluidity of the membrane lipid bilayer and increased osmotic stability of cells (Raccah et al., 1996). It is widely believed that oxidation process and MDA accumulation can contribute directly to changes in the properties of diabetic erythrocytes including decrease in Na⁺, K⁺-ATPase activity, and may cause the development of long-term diabetic complications (Mahesh and Menon, 2004).

During long-term diabetes, oxidant stress may be increased owing to a higher production of ROS and/or deficiency in the antioxidant defense system (Baynes, 1991). The levels of ROS are controlled by antioxidant enzymes, SOD, catalase, Gpx, Gred and nonenzymatic scavengers such as GSH. An impaired radical scavenger function has been linked to decreased activity of enzymatic and nonenzymatic scavengers of free radicals. Reports about the status of antioxidants and scavengers as defense mechanism
during diabetes are very contradictory. Increase, decrease or no change in enzymatic antioxidant concentrations have been reported (Siddiqui et al., 2005; Bukan et al., 2003; Kinalsinski, 2000). Therefore, status of lipid and lipoprotein peroxidation and the status of oxidants, enzymatic and nonenzymatic antioxidants in plasma, erythrocytes and other tissues such as liver of chronic diabetic rats with hyperlipidemia, nephropathy and retinopathy, and other abnormalities of membrane-linked functions are highly important. Furthermore, chronic diabetes may enhance oxidative stress not only through the increased production of ROS but also through weakening the antioxidant defense system. In this context, antioxidant role of serum HDL-complexed paraoxonase (PON)/arylesterase enzyme in the protection of LDL as well as HDL from oxidative modification is noteworthy. In contrast to PON, xanthine oxidase is known to be an important biological source of free radicals. Serum and liver xanthine oxidase activity is significantly increased in diabetes and hyperlipidemia and that free radicals generated in the enzymatic processes are involved in oxidative damage (Desco et al., 2002). Thus, the antioxidants that have both antiradical and xanthine oxidase inhibitory properties may have additional therapeutic benefit in the treatment of diabetes with and without CHD. Finally, risk of diabetic complications including CHD would be further exacerbated by an inadequate dietary intake of antioxidants such as α-tocopherol (vitamin E).

The tocotrienol isomers (α-, β-, γ-, δ-) or naturally occurring analogues of tocopherol isomers (vitamin E) found mainly in cereal grains, rice bran and palm oil. Tocotrienols (T3s) differ from tocopherols (Ts) by possessing three double bonds in phytol side chain. Published reports indicate that both in vitro and in intact membranes including LDL particles T3s exert significantly greater protection against CAD (Packer, 1995; Qureshi et al., 2000). Unlike Ts, T3s have been shown to have an intrinsic hypcholesterolemic activity in animals and humans. The cholesterol lowering effect of T3s was attributed mainly to their down regulation of HMG-CoA reductase -the rate limiting enzyme in the cholesterol biosynthetic pathway (Parker et al., 1993; Minhajuddin et al., 1999; Minhajuddin et al., 2005). The tocopherol isomers do not inhibit cholesterol synthesis and thus do not lower serum cholesterol (Qureshi et al., 1986; Parker et al., 1993). Qureshi and coworkers have demonstrated a strong hypolipidemic effect of tocotrienol rich fraction (TRF)/tocotrienols in various animal
models as well as hyperlipidemic humans (Qureshi et al., 1986; Qureshi et al., 1991a; Qureshi et al., 1997; Qureshi et al., 2000; Qureshi et al., 2001a). Our laboratory has also reported a strong hypolipidemic impact of TRF in normal and hyperlipidemic rats and humans as well as in hyperlipidemic rabbits with accelerated aortic atherosclerosis induced by feeding oxidized cholesterol (Minhajuddin et al., 1999; Minhajuddin et al., 2005; Beg et al., 2000a; Beg et al., 2000b; Beg and Zainuddin, 2003). However, a detailed investigation pertaining to combined hypolipidemic, anti-lipid-/lipoprotein-peroxidative and hypoglycemic as well as antidiabetic impacts of tocotrienols in diabetic patients or diabetic animals has been lacking. There is only one report indicating some hypoglycemic activity of TRF in STZ-induced diabetic rats (Nazaimoon and Khalid, 2002). In this report, feeding of a TRF-rich diet to diabetic rats for 12 weeks caused a significant decrease in blood glucose (32 %) and glycated hemoglobin (gHb, 24 %) levels with no change in serum AGE and MDA levels. However, this hypoglycemic effect of TRF was minimal and both the glucose and gHb levels after TRF treatment were in diabetic range. Recently, our laboratory has reported that TRF administration to type 2 diabetic patients with hyperlipidemia for 60 days was associated with a significant decline in serum total lipids, TC, VLDL-C and LDL-C. However, potential hypoglycemic effect of TRF was not seen in these patients because they were glycemically stable and their base line blood glucose and HbA1 levels were close to normal values (Baliarsingh et al., 2005). In contrast to our results, Nazaimoon et al. (1996) have reported that intake of TRF by type 2 diabetics for 180 days caused no significant change in serum TG, TC, LDL-C, HDL-C and blood HbA1 levels. Statins including Lovastatin are potent competitive inhibitors of HMG-CoA reductase and are highly effective lipid lowering agents. They are universally marketed and used by both nondiabetic and diabetic hyperlipidemic patients with and without CHD. Scattered reports indicate antioxidant properties of Lovastatin in hyperlipidemic-diabetic hamsters (El-Swefy et al., 2000), atherosclerotic rabbits (Chen et al., 1997; Singh et al., 1997) and in vitro as well as ex vivo studies in patients with hypercholesterolemia (Aviram et al., 1992). However, no hypoglycemic effect of Lovastatin has been reported.

Since chronic hyperglycemia coupled with dyslipidemia is responsible for both microvascular and macrovascular diabetic complications, in the present study, we used
STZ-induced diabetic-hyperlipidemic rats. These rats during a 14-week long-term diabetes had a fully developed nephropathy and retinopathy. We have investigated the hypoglycemic, hypolipidemic, antioxidant and antidiabetic impacts of Tocomin and Lovastatin when fed to diabetic-hyperlipidemic rats for 14 weeks and compared with diabetic control rats. The efficacy of feeding 6 mg Tocomin or 0.50 mg Lovastatin/rat/day in preventing the increase in fasting plasma glucose, glycosylated HbA1c, TG, TC, non-HDL-C, VLDL-C, LDL-C, sd-LDL-C, lb-LDL-C, HDL-C, and its subfractions, HDL2-C and HDL3-C levels in diabetic-hyperlipidemic rats was investigated. In addition, quantification of cholesterol and apoB content in LDL and its subfractions, sd-LDL and lb-LDL isolated from plasma of diabetic-hyperlipidemic rats treated with and without Tocomin or Lovastatin has been done. In order to understand the mechanism(s) of lipid lowering actions of Tocomin and Lovastatin in diabetic-hyperlipidemic rats, we have measured the enzymatic activity of hepatic HMG-CoA reductase. The effect of long-term diabetes on plasma total antioxidants, arylesterase activity, plasma and hepatic xanthine oxidase activity; plasma, erythrocytes and hepatic lipid peroxidation products, that is, conjugated dienes, lipid hydroperoxides and TBARS as well as MDA release in intact erythrocytes and membrane associated Na+, K+-dependent ATPase activity was determined. Furthermore, therapeutic role of Tocomin and Lovastatin in the normalization of the above parameters was investigated. Moreover, antioxidant impacts of Tocomin and Lovastatin on base line levels of ex vivo diene conjugation and lag phase time of in vitro Cu2+-induced oxidation of LDL, sd-LDL and lb-LDL both in the presence and absence of glucose was undertaken. In addition, the efficacy of tocotrienols (Tocomin) and Lovastatin in the protection and restoration of the altered enzymatic and nonenzymatic antioxidant defense system in erythrocytes and liver as well as abnormalities of membrane linked functions including histopathological changes in nephropathy and retinopathy of chronic diabetic-hyperlipidemic rats has also been investigated.