Introduction
Cardiovascular disease (CVD) is a global health problem causing half of deaths in developed countries and 30-40% deaths in developing countries. Since the mid-sixties a number of countries including USA, Australia and New Zealand have reported a decline in CVD mortality. However, in developing countries including India, there is a rapid increase in the prevalence of mortality due to CVD (Murray and Lopez, 1996; The World Health Report, WHO. 1999). There are relatively few mortality studies from India, as there is no uniform completion of death certificates and no centralized death registry for CVD (Reddy, 1993). However, the WHO and the World Bank estimate that deaths attributable to CVD have increased in parallel with the expanding population in India, and that CVD now accounts for a large proportion of disability adjusted life years lost (Murray and Lopez. 1996; The World Health Report, WHO 1999). Of all deaths in 1990, approximately 25% were attributable to CVD, compared with 10% from diarrhoeal diseases, 13% from respiratory infections and 8% from tuberculosis (Murray and Lopez, 1996). South Asian (people who originate from India, Sri Lanka, Bangladesh and Pakistan) migrants to the United Kingdom, South Africa, Singapore and North America experience 1.5-4.0 times higher coronary heart disease (CHD) mortality compared with Indigenous populations (Enas et al., 1992). By the year 2015, the proportion of deaths cause by CVD will be almost twice the level in 1985 that is from 19% in 1985 to 35% in 2015 (Lopez, 1993; Khor. 1997). In the Indian urban population, the prevalence of hypertension and coronary artery disease (CAD) is 3-4 times higher than in rural subjects. In South India (Malhotra, 1967; Raman et al., 1993). the prevalence rates are higher in the urban as well as the rural population. CAD is more common in males than females. It seems that differences in diet, life style and the ageing of populations may be important in the pathogenesis of hypertension and CAD in different population groups of Indians (Beegom and Singh, 1995; Singh et al., 1995a). In
the United States alone. CAD accounts for fully one-half of the nearly 1 million deaths each year from cardiovascular disease, and is leading cause of death in both genders (American Heart Association, 1994). Each year, about 1.5 million Americans suffer acute myocardial infarction, and almost all myocardial infarctions are due to the atherosclerosis of coronary heart arteries. Among the two thirds who survive the myocardial infarction, about two thirds do not make a full recovery. In 19 percent of Americans aged 15 years or older who are categorized as disabled, the disability is from CAD or other cardiovascular disease (American Heart Association, 1994).

Risk factor reduction is the primary clinical approach in preventing CAD morbidity and mortality. Epidemiological studies have clearly demonstrated that risk factor such as dyslipidemia, hypertension and the use of tobacco products act in a synergistic manner (Anderson et al., 1991). High levels of total cholesterol, triacylglycerols (TAG), LDL-cholesterol, VLDL-cholesterol, apoB, Lp(a) and low levels of HDL-cholesterol, HDL2-cholesterol, HDL3-cholesterol, apoA-1 are some of the lipid risk factors associated with CAD. Other non lipid risk factors include physical inactivity, obesity, family history of CAD, age, gender, homeostatic factors, homocysteinemia, alcohol consumption and psychological factors. The identification of risk factors provide a means for decreasing CAD risk, through the modification of modifiable risk factors, and for informing treatment decisions, through more accurate determination of overall risk status. The understanding of risk factors and their relationship to the incidence of CAD evolved from prospective epidemiological studies in United States and Europe (Dawber et al., 1957; Report of the Working Group on Arteriosclerosis of NHLBI, 1981; Cooper, 1993; American Heart Association, 1994). These studies identify consistent association between characteristics observed at one point of time in apparently healthy individuals, with the subsequent development of CAD in them. Probably
the single most important contributory factor that has been implicated by various epidemiological studies in the development of CAD, is hypercholesterolemia.

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) 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. 1.1). 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
ACETYL-CoA

Acetoacetyl-CoA Thiolase
Acetoacetyl-CoA Synthase

ACETOACETYL-CoA

HMG-CoA Synthase

ACETOACETATE

HMG-CoA Reductase

MEVALONATE

Mevalonate Kinase

MEVALONATE PHOSPHATE

MEVALONATE PYROPHOSPHATE

HMG-CoA

trans-Methyl glutaconate

ISOPENTYL PYROPHOSPHATE

HMG-CoA

DIMETHYLALLYL PYROPHOSPHATE

cis-Prenyltransferase
trans-Prenyltransferase

GERANYL PYROPHOSPHATE

ISOPENTYL-tRNA

SQUALENE

Squalene Synthetase

FARNESYL PYROPHOSPHATE

DOLICHOL

STEROIDS
BILE ACIDS
LIPOPROTEINS

LANOSTEROL

HAEM A
FARNESYLATED PROTEINS
e.g., Ras, Lamin B

SQUALENE

UBIQUINONE

LANOSTEROL

CHOLESTEROL

Fig 1.1. The biosynthetic pathways of mevalonate, sterols and isoprenoid compounds.
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 precise 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 (Luskey, 1991). 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., 1983a; Brown and Simoni, 1984). HMG-CoA reductase is an approximately 100 kDa 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 Shrewsburg, 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., 1982a; Beg et al., 1982b) 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, feed-back 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; Chan 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), cholestyramine 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; Mitropoulous 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 (Fig. 1.2) (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, 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: cholesteryl 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 (Fig. 1.2). 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 TAG covered by a surface monolayer of phospholipids, free cholesterol and
Fig. 1.2. A model for plasma triacylglycerol and cholesterol transport.  
(Adapted from Biochemistry by D. Voet and J. G. Voet, J. Willey and sons, New York, 1990 ed., p-306)
apoproteins. 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 HDL$_2$ and HDL$_3$. 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 apoA-I and apoAII (Oran et al., 1983; 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 (Kane et al., 1980; Kane, 1983). 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.

Over the last decade three major physiological functions for the plasma apolipoproteins have been identified. (i) Apolipoproteins functions 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 (Narun et al., 1982; 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 (Breckennidge 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 (Davignan et al., 1988; 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 TAG-rich chylomicrons secreted by the intestine. These lipoproteins transport dietary cholesterol and TAG 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 TAG hydrolysis and remodeling of the lipoprotein particles. With TAG 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 (Davignan et al., 1988). ApoE has been proposed to interact and initiate the hepatic uptake of the chylomicron remnants (Davignan et al., 1988; Hertz et al., 1988). The second apoB cascade is a parallel cascade involving TAG 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 triglycerol 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 (Fig. 1.3a). 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) (Fig. 1.3b) 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 (Fig. 1.3c). 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).
Fig. 1.3. Liver LDL receptors control plasma LDL production and uptake. (A), Normal human subjects; (B), Individuals with Familial hypercholesterolemia and (C), Individuals who ingest a high cholesterol diet. (Adapted from Biochemistry by D. Voet and J.G. Voet, J. Willey and sons, New York, 1990 ed, p-306)
Nascent HDL, primarily in the form of phospholipid-apolipoprotein A-I discs, are synthesized both in the human liver and intestine. 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 TAG 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 exchange protein (or lipid transfer protein) 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 (Fig. 1.4). This process is termed as reverse cholesterol transport (Brewer et al., 1971; Eisenberg et al., 1984b). In this proposed model, HDL interacts with a putative HDL receptor (Oran et al., 1983; 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., 1984b).

Atherosclerosis, which is the most common form of arteriosclerosis (hardening of arteries), is characterized by the presence of atheromas. These atheromas or arterial thickenings exude a paste of yellow deposit of almost pure
Fig. 1.4. General overview of "reverse cholesterol transport".
(Adapted from Atherosclerosis and Coronary Artery Disease, edited by V. Fuster, R. Ross and E.J. Topol, Lippincott-Raven Publishers, Philadelphia, 1996, p-71)
cholesteryl esters upon sectioning. Atherosclerosis is a progressive disease and results due to the deposition of intracellular lipids in the smooth muscle cells of the inner arterial wall. These lesions narrow and eventually block the arteries due to the formation of fibrous, calcified plaques. The rough arterial wall promotes the formation of blood clots, which may also block the artery. Due to the blocking of arteries, blood flow stops and causes the death of the deprived tissues. The stoppage of blood flow is known as an infarction. Atheromas mostly occur in the arteries supplying blood to the heart known as coronary arteries. This results in myocardial infarctions or heart attacks, which is the most common cause of death in Western man (Packer and Landvik. 1989). The earliest lesions of atherosclerosis can be found in young children and infants in the form of a lesion called the fatty streak, whereas the advanced lesion, the fibrous plaque, generally appears during early adulthood and progresses with age (Geer et al., 1961; Geer, 1965; Ghidoni and Oniel. 1967; Stary. 1983; Stary et al., 1994; American Heart Association, 1994). Fatty streaks were first observed by Stary (1983), he demonstrated that by the age of ten years, the fatty streaks consisted principally of lipid laden macrophages and smooth muscle cells and appears as yellow coloured area due to large amount of lipid filled foam cells. The bulk of lipid is generally formed by cholesteryl esters, cholesterol derived from lipoproteins.

Stary (1983) also studied fatty streaks in coronary arteries of children and young adults and observed that they were localized at same anatomical sites as the fibrous plaques did in older individuals. Based on this observation he concluded that the fatty streak with age advanced to form the mature fibrous plaque in adults. Chemical analysis of severe atherosclerotic plaques from humans and animal models indicate that on an average, fully developed atheromas are composed of about one half lipid and one half protein. including the cellular and extra cellular proteins, however, there may be great variations in these elements.
Two decades ago, atherosclerosis was considered to be a degenerative process because of the accumulation of lipid and necrotic debris in the advanced lesions, it is, however, now recognized that it is in fact a multifactorial phenomena. The form and content of the advanced lesions of atherosclerosis demonstrates results of three fundamental biological processes; accumulation of intimal smooth muscle cells, together with variable numbers of macrophages and T-lymphocytes. formation of large amount of connective tissue matrix, including collagen, elastic fibers, and proteoglycans by the proliferated smooth muscle cells and accumulation of lipids, principally in the form of cholesteryl ester and free cholesterol within the cells as well as in the surrounding tissues (Ross and Glomset, 1973; Ross and Glomset. 1976; Fuster et al., 1992; Ross and Fuster, 1996).

Jackson and Gotto (1976) have suggested that injury to endothelial cell membrane at arterial wall, may occur upon exposure to chronically elevated levels of LDL due to an increase in the number of cholesterol molecules in plasma membranes of cells, including endothelial cells. Elevation of the cholesterol/phospholipid ratio of endothelial cell plasma membrane can theoretically lead to an increase in the viscosity and decrease in the malleability of endothelial cell surface, which has a critical effect at particular anatomical sites, such as points of bifurcation in the arterial tree. This could lead to inter-endothelial cell separation and retraction particularly at the sites where the blood flow is modified due to the formation of fatty streaks, as has been found in hypercholesterolemic monkeys, swine and humans.

Repeatedly, epidemiological studies have established a strong association between elevated blood cholesterol and CAD. Risk for CAD is increased with increasing plasma cholesterol levels and can be decreased by decreasing plasma cholesterol. One of the important observational study demonstrating a positive
relation between total cholesterol level and CAD mortality was “The Multiple Risk Factor Intervention Trial” (Stamler et al., 1986). It was observed that in Japan and some other Mediterranean countries, where the dietary intake of saturated fat and mean plasma cholesterol level was relatively low, the mortality rate for CAD was also low. As against this, countries like Finland and United States, with high average plasma cholesterol levels showed a higher CAD mortality rate (Keys, 1970). An important interventional study on primary prevention of CAD was the Lipid Research Clinic Coronary Primary Prevention Trial. The hypercholesterolemic subjects of this study received either bile acid sequestrant, cholestyramine or a placebo (LRCP, 1984a). The cholestyramine group, showed significant reductions in LDL-cholesterol. Incidence of non-fatal myocardial infarction and CAD death was also reduced significantly (LRCP, 1984b) in this group. The results of Lipid Research Clinic Coronary Primary Prevention Trial provided the first major clinical substantiation to the lipid hypothesis, and were also the first to give rise to the rule of thumb that a 1% decrease in total cholesterol reduces the incidence of CAD events by 2 to 3%.

Increased plasma levels of three lipoproteins, LDL (Gordon et al., 1977; Castelli et al., 1977), β-VLDL (Mahley, 1979; Brewer et al., 1983) and Lp(a) (Berg et al., 1974; Kostner et al., 1981; Armstrong et al., 1986) and decreased levels of HDL (Castelli et al., 1977; Miller et al., 1977; Naito, 1980; Yaari et al., 1981) have been associated with the development of premature cardiovascular disease. Increased plasma levels of LDL may result from ingestion of excess dietary saturated fat and cholesterol as well as from primary and secondary hyperlipoproteinemias. β-VLDL are abnormal lipoprotein remnant particles present in patients with type-III hyperlipoproteinemia and animals fed a high cholesterol and fat diet (Mahley, 1979; Havel, 1982a; Brewer et al., 1983). β-VLDL remnant lipoproteins have been proposed to be taken up by macrophages by
either the LDL receptor (Koo et al., 1988) or a specific macrophage β-VLDL receptor (Venlenter et al., 1983; Baker et al., 1984). Elevated levels of Lp(a) are genetically determined and associated with an increased risk of premature cardiovascular disease (Berg et al., 1974; Kostner et al., 1981; Armstrong et al., 1986). The mechanism for the potential cellular uptake of Lp(a) and the development of premature cardiovascular disease is not known. Over the past 30 years a considerable body of evidence has accumulated to establish that an elevated concentration of the Lp(a) lipoprotein, commonly referred to as "lipoprotein little a" in plasma is an important independent risk factor for the development of premature cardiovascular disease (Berg et al., 1974; Kostner, 1981; Armstrong et al., 1986). Lp(a) levels in plasma range from less than 1 to more than 100 mg/dl. Approximately 20 per cent of the populations have levels above 30 mg/dl; this is associated with a twofold increase in the relative risk of coronary atherosclerosis. With elevations of both Lp(a) and LDL, the relative risk of vascular disease increases to approximately fivefold (Armstrong et al., 1986).

An inverse association has been established between HDL-cholesterol level and CAD incidence in numerous epidemiological studies. For example, in the Framingham Heart Study, men and women with HDL-cholesterol of 35 mg/dl or less had an eight fold increase in CAD incidence compared with men and women with HDL-cholesterol of 65 mg/dl or greater (Gordon et al., 1977). Women have significantly higher plasma levels of HDL₂ than men (James and Pometta, 1990); increased levels of these larger, less dense particles may be partially responsible for the relative cardioprotection seen in premenopausal women. Each 1 mg/dl increase in HDL-cholesterol is estimated to decrease CAD risk 2 per cent in men and 3 per cent in women (Gordon et al., 1989). The low HDL in men with clinically significant CAD is due to a reduction in both HDL₂ and HDL₃; however, the reduction in HDL₂ is greater than that in HDL₃ (Miller et al., 1981; Laakso et
al., 1985; Wallentin and Sundin, 1985; Hamsten et al., 1986). These studies support the concept that HDL$_2$ is a better predictor of coronary artery disease than is HDL$_3$ (Drexel et al., 1992). The question, however, as to whether quantitation of HDL$_2$ as a screening test is superior to total HDL-cholesterol has not been definitively answered due to variability in results of the individual reported studies.

The results of the studies in which apoA-I was evaluated as a discriminator for CAD also do not permit a definitive conclusion to be drawn as to whether apoA-I is a better discriminator than HDL-cholesterol. In the majority of patients with angiographically documented CAD or survivors of myocardial infarction, plasma apoA-I concentrations were lower than control groups (Brunzell et al., 1984; Miller, 1987; Alauporic et al., 1988). In some studies apoA-I has been shown to be a better discriminator than lipids and lipoprotein cholesterol levels in identifying patients with CAD, while in other studies the apoB/apoA-I ratio appeared to be an even more powerful predictor than the individual lipoproteins (Brunzell et al., 1984; Miller, 1987; Alauporic et al., 1988). However, in other studies HDL-cholesterol was found to be a better predictor than apoA-I (Brunzell et al., 1984; Miller, 1987; Alauporic et al., 1988).

The relation between plasma TAG and CAD is not as well established as the relation between plasma cholesterol and CAD. Epidemiological evidence, however, suggests that TAG plays an important role in determining CAD risk. In Helsinki Heart Study (Manninen et al., 1992) it was established that the relative risk for cardiac events was significantly more in the subgroup with a higher TAG and LDL cholesterol: HDL cholesterol ratio as compared to the subgroup with lower TAG and LDL cholesterol: HDL cholesterol ratio. Diabetes is frequently associated with hypertriglyceridemia, and is often combined with reduced HDL-cholesterol (Haward, 1987). Recently, there has been increasing evidence of an association between TAG and increased risk of cardiovascular disease (Austin,
1991), a risk that is especially high in subjects with low HDL-cholesterol (Castelli, 1986). One of the major causes of hypertriglyceridemia with low HDL-cholesterol in diabetes is the decrease in lipoprotein lipase activity, an enzyme that plays crucial role in both TAG removal and HDL-cholesterol production, due to insulin deficiency, because lipoprotein lipase is known to be an insulin-dependent enzyme (Garfinkel et al., 1976; Murase et al., 1981). Elevated levels of plasma apoB and LDL cholesterol may be due to dietary excess, genetic hypercholesterolemias or other diseases. Approximately 10 per cent of the patients with hypercholesterolemia have a monogenic disease causing elevated plasma LDL levels. The monogenic disease, familial hypercholesterolemia, is an autosomal codominant disease characterized clinically by tendon and tuberous xanthomas, arcus, xanthelasma and premature cardiovascular disease (Brown and Goldstein, 1986). The molecular defect in familial hypercholesterolemia is one of several mutations in the gene coding for the LDL receptor (Brown and Goldstein, 1986). The deficiency of the LDL receptor leads to a decreased rate of removal of plasma LDL. The reduction in the number of LDL receptors has been previously demonstrated to be proportional to the elevated levels of plasma LDL (Sprecher et al., 1985). The frequency of familial hypercholesterolemia is approximately 1 in 500 and the heterozygotes and homozygotes have plasma LDL cholesterol levels >250 mg/dl and >750 mg/dl, respectively. Plasma apoB levels are usually greater than 140 mg/dl. The clinically important complication of familial hypercholesterolemia is premature atherosclerosis. In homozygous patients coronary and myocardial infarctions are frequent in young adults. For heterozygous men, the chance of having a myocardial infarction is approximately 5 per cent by age of 30, 51 per cent by the age of 50 and 85 per cent by age of 60.

Familial combined hyperlipidemia is probably the most frequent monogenic dyslipoproteinemia (frequency 1 in 300) in the population associated with an
increased risk of premature cardiovascular disease (Goldstein et al., 1973; Ross et al., 1973). Clinically the patients may have arcus and xanthelasmas; however, tendon xanthomas are rare and this may be used as a diagnostic clue to distinguish these patients from patients with familial hypercholesterolemia. Plasma cholesterol and TAG levels are generally moderately elevated. A characteristic feature of combined hyperlipidemia is that the lipoprotein elevation is variable and may change from elevated LDL (phenotype IIa) to VLDL and LDL (phenotype IIb) and VLDL (phenotype IV). In addition, several different phenotypes may be observed in a single family. In contrast to familial hypercholesterolemia, the cholesterol levels may not be elevated in childhood and the hyperlipidemia frequently is not expressed until after age 30. The molecular defect(s) in combined hyperlipidemia has not been elucidated. In the patients studied, the characteristic metabolic defect is the over production of apoB-containing lipoproteins (Tenz et al., 1986). The LDL present in patients with combined hyperlipidemia is abnormal in hydrated density and lipid composition. The “dense LDL” characteristic of combined hyperlipidemia has a hydrated density of approximately 1.055 gm/ml and has a ratio of LDL cholesterol to LDL apoB of <1.3 as compared with a normal ratio of >1.3 (Sniderman et al., 1980; Krauss et al., 1988; Musliner and Krauss, 1988). The LDL apoB level in these patients is usually >120 mg/dl. The presence of “dense LDL” may be an important causative factor in the development of atherosclerosis observed in these patients. Elevated plasma level occur in type III hyperlipoproteinemia and in experimental animals fed diets high in cholesterol and saturated fat (Mahley, 1979; Havel, 1982a; Brewer et al., 1983). Epidemiological studies have established a correlation between the apoE phenotype and alterations in the plasma level of remnant lipoproteins, apoB and LDL cholesterol (Davignan et al., 1988).
Rabbits have been extensively utilized as an ideal model of atherosclerosis because of its size, easy manipulations and extraordinary response to atherosclerosis. In mammalian nonprimate models rabbits and hares have been known to be best in dietary induction; lipoprotein metabolism (Watanable hyperlipoproteinemia strain); dietary and drug intervention; immune complexes; clonal characteristic of lesion (Shore and Shore 1976). Although rabbit is the first and most frequently used animal at all point of atherosclerosis research (Shore and Shore 1976), but many investigators have expressed doubts as the usefulness of this animal model, because the lesions are result of extreme hyperlipidemic conditions. Recently, WHHL rabbit, an excellent model, which can serve as counterpart of homozygous familial hypercholesterolemia in humans, has become available (Watanabe, 1980; Havel et al., 1982b; Buja et al., 1983). Hepatic cholesterol synthesis is found to be by far the highest in the rat. Whereas in the rabbit and man the rate of cholesterol synthesis was 20% and 16%, respectively, in comparison to the rat. Similarly, hepatic ACAT activities tend to be high in the rat, low in the rabbit and man (Suckling et al., 1986). Historically much of the work has used cholesterol fed rabbits, an approach that has received strong criticism (Stehbens et al., 1986). Cholesterol feeding in the rabbits leads to massive hypercholesterolemia characterized by a high VLDL concentration. In contrast, in the WHHL rabbits the hypercholesterolemia is due to increased LDL, as it is in many human hypercholesterolemics. However, feeding regimes have been advised that are less open to such concerns (Bocan et al., 1991). One of the problems of working with fat and cholesterol fed rabbits has been the high variability between individual animals, is not fully understood. The animals appear to maintain a low intracellular cholesterol flux even when on a high cholesterol diet. One study (La Ville et al., 1989), has shown that the HMG-CoA reductase inhibitor, lovastatin, can prevent the formation of atherosclerotic lesions in this animal. Some studies
have combined interest in atherosclerosis with diabetes, Arbeeny and Bergquist. 1991, showed that alloxan diabetic rabbits fed on a diet containing casein and coconut oil developed hypercholesterolemia, which could be controlled by the HMG-CoA reductase inhibitor, provastatin. HMG-CoA reductase inhibitors have also been shown to be effective in controlling hypercholesterolemia in human type II diabetics. Rabbits have been widely used as a model for testing hypolipidemic and antiatherosclerotic drugs. In the past few years, drugs, some of which are not primarily hypolipidemics, have been tested in rabbits for antiatherosclerotic activity, for example calcium channel blockers, cholesteryamine and ACAT inhibitors (Weinstein and Heider, 1989; Suckling 1989; Subbiah et al., 1987). Finally, report has appeared of a transgenic rabbit containing a human apoA-I on antisense gene intended as a model for the genetic direction of atherogenic disturbances of lipid metabolism (Perevozhikov et al., 1990). In all the animals containing the human gene the lipoprotein profile was shifted to an atherogenic phenotype with increase in plasma cholesterol and triglyceride concentration.

Recent studies in nonhuman primates have demonstrated injury by free radicals as another possible mechanism of initiation of atherogenesis, which was protected by the antioxidant, probucol (Sasahara et al., 1994). The fact that probucol prevents the formation of fatty streaks and suppresses the inflammatory processes required for lesion development bears evidence to this effect. These studies have provided new insights into the role of oxidation and other chemical modifications of LDL, such as glycation in the process of atherogenesis. In particular, the oxidative modification of LDL enhances its atherogeneity (Steinberg, 1988; Duthie et al., 1989; Palinski et al., 1989). The etiology of cardiovascular disease is complex and multifactorial, but there is substantial evidence that oxidized lipid in the diet play an important role in atherosclerosis (Staprans et al., 1998; Rong et al., 1999; Liu et al., 2000). Nearly all natural fats
oxidized when exposed to air, light, moisture, particularly warm temperature and develop an unpleasant odour and taste. This happens due to formation of peroxides at the double bonds of the unsaturated fatty acids. Peroxidation of lipids exposed to oxygen is responsible not only for deterioration of foods, oils, fats, but also for damage to tissues in vivo, where it may be the cause of atherosclerosis, cancer, inflammatory diseases and ageing etc. The deleterious effects are initiated by free radicals ROO', RO*, OH* produced during peroxide formation from fatty acids containing double bonds. Lipid peroxidation is a chain reaction providing a continuous supply of free radicals that initiates further peroxidation. Lipid peroxidation usually begins with the abstraction of a hydrogen atom from an unsaturated fatty acid resulting in the formation of a lipid radical (Poyer and Stanley, 1975). The rearrangement of the double bonds results in the formation of conjugated dienes. Attack by molecular oxygen produces a lipid peroxy radical, which can either abstract a hydrogen atom from an adjacent lipid to form a lipid hydroperoxide, or form a lipid endoperoxide. The formation of lipid endoperoxides in unsaturated fatty acids containing at least three methylene interrupted double bonds can lead to the formation of malondialdehyde as a breakdown product (Fig. 1.5). Microsomes isolated from liver have been shown to catalyze an NADPH-dependent peroxidation of endogenous unsaturated fatty acid in the presence of ferric ions and metal chelators such as ADP or pyrophosphates. Microsomal membranes are particularly susceptible to lipid peroxidation owing to the presence of high concentrations of polyunsaturated fatty acids. Poyer and McCay (1971), have demonstrated that both microsomal membranes and phosphate buffer contain sufficient contaminating iron to facilitate NADPH-dependent microsomal lipid peroxidation. Free radicals are molecule that have lost an electron and try to replace by reacting with other molecules. Oxidative damage can occur when chemical processes within the body generate free radicals. Some
Fig. 1.5. Lipid peroxidation.
Free radicals are highly reactive and can interact and damage functionally important molecules such as DNA, lipid membrane constituents, structural proteins and impairing our immune system. We are most often subjected to free radicals from air pollution, exposure to radiation and toxic chemicals, cigarette smoke, UV radiation, fried and burnt foods, food and water contaminants such as pesticides. Converting food or stored fats to energy generates free radicals in our body as well. Cigarette smoke contains vast amounts of free radicals, that is, $10^{14}$ free radicals per inhalation, which can directly or indirectly initiate and propagate the process of lipid peroxidation.

It has been demonstrated earlier that oxidized fatty acids in the diet play a significant role in lipoprotein oxidation. In rodents (Staprans et al., 1993a; Staprans et al., 1993b; Staprans et al., 1996a) and humans (Staprans et al., 1994), oxidized fatty acids in the diet are absorbed by the small intestine and incorporated into chylomicrons. In rodents, oxidized dietary fatty acids are also incorporated into the endogenous serum VLDL+LDL fraction (Staprans et al., 1993b). The levels of oxidized chylomicrons and VLDL+LDL directly correlate with the quantity of oxidized lipids in the diet. Furthermore, Staprans et al. have shown that oxidized lipids in the diet are delivered to the liver via chylomicrons, incorporated into VLDL, and resecreted into the circulation, thereby providing a mechanism by which dietary oxidized lipids can affect the oxidative state of endogeneous lipoproteins (Staprans et al., 1996a). It has been demonstrated that oxidized lipids in the diet are atherogenic. Feeding a diet enriched in oxidized fatty acids to cholesterol-fed rabbits resulted in a significant increase in fatty streak lesions in the aorta (Staprans et al., 1996b). Similar to fatty acids, cholesterol also undergoes free radical-mediated oxidation via hydroperoxide formation, resulting in the production of numerous oxygenated derivatives (oxidized cholesterol or oxysterols). There is growing evidence that cholesterol oxidation products
contribute to the development of atherosclerosis (Smith 1996; Staprans et al., 1998; Rong et al., 1999). Studies specifically addressing this issue have shown that cholesterol oxidation products possess several characteristics that may promote atherosclerosis. These include cytotoxic/apoptotic potential to vascular cells such as fibroblasts (Sevanian and Peterson, 1986), endothelial cells (Sevanian et al., 1991; Sevanian et al., 1995; Palladini et al., 1996), and smooth muscle cells (Peng et al., 1979; Hughes et al., 1994; Nishio and Watanabe, 1996); impairment of vascular endothelial barrier function (Boissonneault et al., 1991a; Boissonneault et al., 1991b); inhibition of endothelial NO release (Deckert et al., 1998) and arterial relaxation (Deckert et al., 1997); inhibition of cholesterol synthesis or utilization (Tamasawa et al., 1997; Taylor and Kandutsch, 1985); perturbation of intracellular cholesterol trafficking (Kilsdonk et al., 1995; Fielding et al., 1997; Gelissen et al., 1996); and activation of acyl CoA: cholesterol acyltransferase (ACAT) activity (Cheng et al., 1995; Brown et al., 1975). Cholesterol oxidation products are abundant in oxidatively modified LDL (Hodis et al., 1994) 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 evidence linking circulating cholesterol oxidation products to early vascular lesion formation is only recently becoming available (Mahfouz et al., 1997; Staprans et al., 1998; Rong et al., 1999). These results demonstrate that diets containing oxidized fatty acids or oxidized cholesterol accelerate atherosclerotic lesions. It is well established that due to processing, heating, frying/deep frying or prolonged storage, the Indian as well as the Western diet contains large quantities of oxidized polyunsaturated fatty acids and oxidized cholesterol, that could constitute a risk factor for cardiovascular disease. Oxidized lipoproteins especially oxidized low density lipoproteins have been involved in the pathogenesis of atherosclerosis and atherosclerotic lesions contain oxidized LDL (Steinberg et al., 1989; Witztum and
Steinberg, 1991; Esterbauer et al., 1992; Yla-Herttuala et al., 1989). The oxidation of LDL can be mediated by various cell types (Witztum and Steinberg, 1991; Esterbauer et al., 1992). Recently, Liu et al. (2000), have demonstrated that not only LDL but also VLDL and HDL were oxidatively modified in vivo in the patients with endogenous hypertriglyceridemia and in the rabbits fed with a high cholesterol diet. According to oxidative modification hypothesis, LDL initially accumulates in the extracellular subendothelial space of arteries and, through the action of resident vascular cells, is mildly oxidized to a form known as minimally modified LDL (Navab et al., 1996). This minimally modified LDL induces local vascular cells to produce monocyte chemotactic protein 1 and granulocyte and macrophage colony stimulating factors, which stimulate monocyte recruitment and differentiation to macrophages in arterial walls (Parhami et al., 1993). The accumulating monocytes and macrophages stimulate further peroxidation of LDL (Fig. 1.6). The products of this reaction make the protein component of LDL (apolipoprotein B-100) more negatively charged. By virtue of its increased negative charge, this completely oxidized LDL is recognized by scavenger receptors on macrophages and internalized to form so called foam cells (Henriksen et al., 1981). In contrast to the uptake of unoxidized (native) LDL by the LDL receptor on macrophages, the uptake of oxidized LDL by the scavenger receptor pathway is not subject to negative feedback regulation and thus results in massive uptake of cholesterol (from oxidized LDL) by the macrophages.

In addition to promoting the formation of foam cells, oxidized LDL has direct chemotactic activity for monocytes (Quinn et al., 1988), and stimulates the binding of monocytes to the endothelium (Frostegard et al., 1991). Once monocytes cross the endothelial layer, they become trapped in the subendothelial space, partly because oxidized LDL inhibits their egress from the arterial wall (Quinn et al., 1987). Oxidized LDL is also cytotoxic to vascular cells (Schwartz et
Fig. 1.6. Early events in atherogenesis
al., 1991; Cathcart et al., 1985), thus promoting the release of lipids and lysosomal enzymes into the intimal extracellular space and enhancing the progression of atherosclerotic lesions (Schwartz et al., 1991). The oxidative modification hypothesis is supported by evidence that LDL oxidation occurs in vivo and contributes to the clinical manifestations of atherosclerosis. Antibodies raised against oxidized LDL react with atherosclerotic lesions, but not with normal arterial segments (Palinski et al., 1989). LDL extracted from human atherosclerotic lesions, but not plasma-derived LDL, resembles LDL that has been oxidatively modified in vitro (Yla-Herttuala et al., 1989). Patients with carotid atherosclerosis have higher levels of autoantibodies to oxidized LDL than do age-matched normal subjects (Salonen et al., 1992). Plasma concentrations of immunoreactive oxidized LDL are higher in patients with acute myocardial infarction than in normal subjects (Holvoet et al., 1995). Thus, oxidative modification of LDL appears to have an important role in foam cell formation and atherogenesis (Fig. 1.6). This link between the oxidation of LDL and atherogenesis provides a convenient and simple rationale for the beneficial effect of antioxidants on the incidence of coronary artery disease. A number of clinical studies and studies in animals have explored this link between LDL oxidation and atherogenesis. Several studies have investigated the ability of various antioxidant compounds to prevent atherogenesis in animals. Probucol is a lipid soluble cholesterol lowering drug with potent antioxidant properties (Reaven et al., 1992). When fed to WHHL rabbits, it inhibits the formation of atherosclerotic lesions independently of its cholesterol lowering properties (Carew et al., 1987), and LDL derived from rabbits given probucol is more resistant to oxidation than LDL from control rabbits (Kita et al., 1987). These results support the concept that the antiatherogenic effect of probucol involves an antioxidant mechanism. Probucol also reduces the formation of atherosclerotic lesions in cholesterol fed monkeys,
and there is an inverse relation between the formation of lesions and the resistance of isolated LDL to oxidation (Sasahara et al., 1994). Similarly, cholesterol fed rabbits treated with N,N'-diphenyl-phenylenediamine, a synthetic antioxidant that is structurally distinct from probucol, have less atherosclerosis and LDL that is more resistant to oxidation than do untreated rabbits (Sparrow et al., 1992). Vitamin E reduces atherosclerosis in WHHL rabbits (Williams et al., 1992) and in cholesterol fed hamster (Parker et al., 1995), although in WHHL rabbits this effect is confounded by a reduction in serum cholesterol concentrations (Williams et al., 1992). Thus, a number of studies in animals have demonstrated that antioxidants inhibit the development of atherosclerosis, and in many of these studies, antioxidant treatment enhanced the resistance of LDL to oxidation. This association between reduced atherosclerosis and increased resistance of LDL to oxidation provides support for the oxidative modification hypothesis of atherosclerosis. Many clinical studies have investigated the effect of antioxidative supplementation in humans on LDL oxidation ex vivo (Keaney and Frei, 1994a). The administration of lipid soluble antioxidants such as α-tocopherol (Reaven et al., 1993) or probucol (Reaven et al., 1992) is associated with their incorporation into LDL and concomitant increase in the resistance of LDL to oxidative modification. In contrast, supplementation of β-carotene does not protect LDL from oxidation, despite its accumulation within the particle (Reaven et al., 1993; Gaziano et al., 1995). Even though α-tocopherol has been labelled as the most efficient chain breaking antioxidant, tocotrienols are known to be more potent antioxidant than tocopherols (Kamat et al., 1997). Tocotrienols have been shown to have greater free radical scavanging properties as cell membrane constituents than tocopherols (Serbinova et al., 1991). Tomco et al.(1995), reported that tocotrienols significantly decreased plasma lipid peroxidation in patients with hyperlipidemia and carotid atherosclerosis with no change in their lipid and
lipoprotein parameters. Ascorbic acid (vitamin C) also protects LDL against oxidation but is not incorporated into LDL, because it is water soluble (Retsky et al., 1993). In the majority of studies linking antioxidants with decreased atherosclerosis, the atherogenic diet and antioxidant therapy were introduced simultaneously (Sasahara et al., 1994; Sparrow et al., 1992; Williams et al., 1992), or at a very early stage (Carew et al., 1987, Kita et al., 1987). Such studies focus on the initiating events in atherogenesis, and the decrease in LDL oxidation by antioxidants could explain the reduction in early formation of foam cells and plaque. Although in humans supplementation with lipid soluble antioxidants protects LDL against ex vivo oxidation (Keaney and Frie, 1994a), prevention of the initiating events in atherosclerosis may not be the principal mechanism responsible for reduction in the clinical manifestations of atherosclerosis that has been linked to higher antioxidant intake (Fig. 1.7).

Oxidized LDL is toxic to vascular cells (Morel et al., 1983), and the cytotoxic potency of oxidized LDL has been linked to its content of lipid peroxidation products (Morel et al., 1983; Hughes et al., 1994). Inhibiting LDL oxidation should therefore decrease the cytotoxicity of LDL in arterial walls. In fact, oral supplementation with α-tocopherol increases the resistance of LDL to oxidation and lowers the cytotoxicity of this oxidized LDL toward endothelial cells (Belcher et al., 1993). Endothelial denudation induced by oxidized LDL may also be an important trigger of coronary thrombosis and acute coronary events (Fuster et al., 1992). suggesting that improved resistance of LDL to oxidation will limit the oxidized LDL-mediated cytotoxicity that contributes to instability of lesions, rupture of plaques, and coronary thrombosis events. In the presence of oxidized LDL, the physiologic action of nitric oxide is impaired, which might contribute to platelet adhesion and vasospasm that are involved in the pathogenesis
Fig. 1.7. LDL-specific and tissue-specific mechanisms of antioxidant action
(Adapted from The New England Journal of Medicine, 1997, Vol. 337, p. 414)
of acute coronary syndromes (Levine et al., 1995). Enhanced protection of LDL against oxidation is associated with a number of vascular effects that would be expected to reduce the clinical activity of coronary artery disease: reduced plaque rupture, platelet adhesion, and vasospasm (Fig. 1.7). In addition to improving the nitric oxide action by reducing the oxidation of LDL, antioxidants are beneficial to vascular function through other mechanisms. Cholesterol fed rabbits has reduced endothelium derived nitric oxide-mediated vascular relaxation. This reduction can be prevented by the administration of α-tocopherol (Keaney et al., 1993; Andersson et al., 1994; Steward-Lee et al., 1994), β-carotene (Keaney et al., 1993), or probucol (Keaney et al., 1995; Simon et al., 1993). One simple explanation for these observations would be that LDL oxidation is prevented in vivo. The aforementioned effects of antioxidants on vascular function (Keaney et al., 1993; Keaney et al., 1994; Keaney et al., 1995; Simon et al., 1993; Andersson et al., 1994; Steward-Lee et al., 1994) are most likely related to the presence of these compounds in the vascular wall, where they are known to accumulate.

Monocytes and macrophages in atherosclerotic lesions express matrix metalloproteinases that degrade components of extracellular matrix (Galis et al., 1995), thus weakening atherosclerotic plaques and increasing the likelihood of plaque rupture. Upto 98% of patients with acute coronary thrombosis have a thrombus in the arterial lumen in direct communication with macrophage rich regions of plaque (Friedman and Bovenkamp, 1966). The recruitment of monocytes and macrophages into these regions is regulated, in part, by endothelial cells through the selective expression of specific cellular adhesion molecules (Collins, 1993). Similarly, monocytes derived from subjects treated with α-tocopherol adhere to activate endothelium to a lesser extent than do monocytes from normal subjects (Devaraj et al., 1996). These data support the concept that direct cellular action of α-tocopherol has a role in inhibiting the adhesion of
monocytes to endothelium and in promoting plaque stability. The cellular content of antioxidants is an important determinant of cellular injury from oxidized LDL. Endothelial cells and macrophages exposed to oxidized LDL rapidly become necrotic (Kuzuya et al., 1991; Reid and Mitchinson, 1993). In contrast, endothelial cells and macrophages loaded with α-tocopherol or probucol are resistant to the cytotoxic effects of oxidized LDL (Kuzuya et al., 1991). Thus, cellular antioxidant status is an important determinant of the extent of cell injury resulting from oxidized LDL that contributes to plaque instability (Fig. 1.7). Platelets have an important role in the pathogenesis of atherosclerosis and coronary thrombosis (Schwartz et al., 1991), and there is now convincing evidence that supplemental concentrations of α-tocopherol inhibit platelet function. The administration of 400 to 1200 IU of α-tocopherol per day for two weeks to normal subjects was associated with a marked reduction in the sensitivity of platelets to activation by platelet agonists such as arachidonic acid and phorbol ester (Freedman et al., 1996). This effect of α-tocopherol is related to inhibition of protein kinase C stimulation rather than to its antioxidant activity (Freedman et al., 1996). Similarly, the inhibition of protein kinase C stimulation by α-tocopherol also prevents the proliferation of smooth muscle cells (Boscoboinik et al., 1991). Smooth muscle proliferation is a major component of restenosis after coronary artery balloon angioplasty, and recent evidence indicates that vascular antioxidant content may modulate this process. These results support the view that incorporation into tissues may be important in the therapeutic action of antioxidant.

As humans evolved to use oxidative metabolism, many mechanisms have been developed to control this process and minimize random free radical oxidation. Firstly, oxidative metabolism is compartmentalized for example, in the mitochondria. Secondly, molecular oxygen and its reactive free radical species are tightly bound to enzymes, as in case of the cytochrome systems during oxidative
phosphorylation. Thirdly, in order to prevent free radical formation, transition metals like copper and iron, which in the free form catalyze free radical formation, are tightly bound to transport and storage proteins. Fourthly, several enzymes exist within the cells to neutralize free radicals. Superoxide dismutase catalyses the transformation of superoxide radicals to hydrogen peroxides. Similarly, catalase and glutathione dismutase neutralize hydrogen peroxide and fatty acid radicals. Finally, damage caused by oxidation can be repaired by specific enzymes.

If, however, inspite of the availability of all the above mentioned protective mechanisms against oxidative damage, due to any reason, the free radical peroxidation of LDL lipids occurs, it results in numerous structural changes, all depending on a common initiating event i.e., peroxidation of polyunsaturated fatty acids on LDL (Jurjens et al., 1987; Steinbrecher et al., 1990). Also, all the major cells of arterial wall (endothelial cells, macrophages, smooth muscle cells) can oxidatively modify LDL. Both forms of oxidized LDL are taken up more avidly by the macrophages than the LDL itself and this avidity appear to be facilitated by scavenger receptor mechanism (Jurjens et al., 1987; Steinbrecher et al., 1990). The biological effects of oxidized LDL reported to date could contribute to initiation and progression of atherosclerotic process. Oxidized and partially oxidized LDL itself or the oxidized products derived from it can have a number of deleterious effects, including chemotaxis for monocytes (Qureshi et al., 1991a), inhibition of nitric oxide mediated relaxation of coronary tone (Schaefer et al., 1985). To summarize, recognition of uncontrolled uptake of oxidized LDL by macrophages leads to a chain of biochemical events, which can result in the formation of foam cells, fatty streaks and later atherosclerosis.

Pharmacological control over cholesterol biosynthesis has long been sought as a means for regulating the amount of cholesterol in the blood and for the prevention and treatment of atherosclerosis (Kolata, 1983). Probucol has been
reported to effectively reduce plasma cholesterol in human and a number of animal species (Miettinen and Toinonen, 1975; Martz, 1979; Sunson et al., 1981). 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 of 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 (Sunson et al., 1981) 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.

Fibrates are orally active compounds with relative long plasma half life. Among fibrates, clofibrates, with combined maximal effectiveness and minimal toxicity in the initial screen, were widely used in the management of hyperlipoproteinemia in humans. More recently, however, due to questionable activity in the secondary prevention of atherosclerosis (Coronary Drug Project, 1975) and to the observation of untoward effects in primary prevention (Committee of Principal Investigators, 1978), the use of clofibrates has become limited. Among side effects, proliferation of peroxisomes in rodents has been a key target for chemical and pharmacological studies. A number of clinical trials have
constantly shown that clofibrate reduces plasma TAG levels substantially, affecting both VLDL and LDL associated TAG. This reduction is more evident in hypertriglyceridemic patients but less marked in normotriglyceridemic subjects (Hunninghake et al., 1981; Crous and Grundy, 1981).

Halofenate, structurally related to clofibrate, is hypolipidemic and hypouricemic (Sirtory et al., 1972) and is effective in lowering serum TAG in rats (Kovanen et al., 1981). Bezafibrate, a fibric acid derivative, has been reported to lower plasma TAG in hypertriglyceridemic patients (Eisenberg et al., 1984b) and was effective in reversing most if not all, of the abnormalities in lipoprotein compositions, structure and function detected in these patients. Fenofibrate, another analogue, is more potent than the previously described compounds, being fully active at a daily dose of 300 mg (Rossner and Oro, 1981). It is markedly effective in patients with type II and IV hyperlipidemias (Franceschini et al., 1985) as well as in subjects with familial combined hyperlipidemia (Weisweiler et al., 1984) in decreasing total plasma cholesterol and TAG levels. However, HDL-cholesterol levels were unchanged in type II and combined hyperlipidemia (Malmendier and Delcroise, 1985). The mechanism of hypolipidemic effect of fenofibrate is different from that of parent compound involving its effect on lipoproteins as seen in subjects with familial hypercholesterolemia as well as combined hyperlipidemia.

Nicotinic acid is possibly the oldest lipid lowering drug. Large doses of this drug rapidly reduce plasma TAG by lowering VLDL in normal and sucrose diet fed mice (Oliver et al., 1988). The reduction in TAG was reported to be due to decreased production of VLDL (Grundy et al., 1981). To overcome the difficulty of obtaining adequate compliance with nicotinic acid treatment, because of drug’s numerous side effects, several analogues and derivatives have been tested but no clear advantages have been gained over the parent drug (Cretaldi et al., 1988).
An alternate approach to the control of cholesterol level is the use of bile sequestrant resin, such as cholestyramine. The mode of action of bile acid binding resin is apparently fairly simple: bile acids, which are bound to these resins in the intestinal lumen, are not reabsorbed, and are excreted with faeces. These sequestrant agents thus interfere with the enterohepatic circulation of bile acids, and since bile acids are synthesized in the liver from cholesterol, cholesterol catabolism is enhanced. After prolonged treatment hypocholesterolemia is observed. Homozygous type II patients are insensitive to cholestyramine inspite of increased level of faecal bile acids (Moutafis et al., 1971). The LDL pathway receptor plays a role in the action of bile acid binding resin (Shepherd et al., 1980).

An encouraging development in the treatment of hypercholesterolemia has been the introduction of a new class of fungal derived compounds that are potent competitive inhibitors of HMG-CoA reductase. These drugs are extremely effective in lowering plasma concentrations of LDL-cholesterol. The development of these specific inhibitors of HMG-CoA reductase has considerably widened the therapeutic opportunities in hypercholesterolemic patients. Compactin and lovastatin (mevinolin) are potent competitive inhibitors of HMG-CoA reductase. The molecular structures closely resemble the HMG moiety of HMG-CoA, and the enzyme HMG-CoA reductase binds both the compounds with high affinity (Paolletti and Poli, 1987). These drugs have been used to reduce plasma cholesterol levels in many animal species (Alberts et al., 1980; Kovanen et al., 1981; Tobert et al., 1982). In clinical studies, lovastatin and compactin effectively reduce plasma LDL in normal (Tobert et al., 1982) as well as in subjects with heterozygous familial hypercholesterolemia (Bilheimer et al., 1983). A compensatory increase in the receptor mediated catabolism of LDL also occurs (Grundy and Bilheimer, 1984). In patients with type IIa and type IIb hypercholesterolemia, reduction in serum cholesterol was reported after lovastatin
Goldstein and Brown (1984a) proposed that statins (compactin, lovastatin and simvastatin) lower plasma LDL cholesterol by inhibiting cholesterol biosynthesis and increasing the number of LDL receptors. Although an increase in HDL levels has also been reported in several studies (Hoeg et al., 1986; Mol et al., 1988), others (Mabuchi et al., 1981; Illingworth and Sextan, 1984) have found no change in HDL levels.

The mechanism of action of lovastatin differs from that of probucol (Helve and Tikkanen, 1988). Lovastatin therapy resulted in the increase of both HDL and HDL₂-cholesterol whereas with probucol HDL-cholesterol was markedly decreased mainly because of reduction in HDL₂. Triacylglycerols remain unaltered during lovastatin treatment and no significant changes in lipase activity were observed indicating that these enzymes were not involved in its action. Recently, other forms of statins such as atorvastatin, pravastatin and fluvastatin have been introduced in the market as lipid lowering drugs. Out of these statins, including lovastatin and simvastatin, atorvastatin is most effective as a lipid lowering agent. Daily intake of 10 to 80 mg of atorvastatin by patients with primary hypercholesterolemia (heterozygous familial and nonfamilial) and mixed dyslipidemia was associated with a 39-60% decline of plasma LDL-C and 19-37% in TG levels. HDL-C level was increased by 5-9%. However, all the statins known to exhibit host of side effects (Assmann et al., 1998; Haffner, 1998).

3-Hydroxy-3-methylglutaric acid (HMG), which is formed by the hydrolysis of HMG-CoA catalysed by HMG-CoA hydrolase in liver, is also known to inhibit cholesterogenesis between HMG-CoA and mevalonate (Rabinowitz and Gurin, 1954) and competitively inhibit the enzyme HMG-CoA reductase (Fimognari and Rodwell, 1965). The hypolipidemic activity of HMG has been studied in rats (Yusufzai and Siddiqui, 1976a, 1977; Francesconi et al., 1987), rabbits (Lupien et al., 1973), hamster (Padova et al., 1982) and humans (Lupien et
A support to the hypolipidemic action of HMG is also obtained from the observation that low incidence of CHD in Massai tribesman is related to their high consumption of cow’s milk (Richardson, 1978), known to contain HMG (Man, 1977).

Inhibitors of HMG-CoA reductase block synthesis of cholesterol in the liver, thereby triggering compensatory reactions that lead to a reduction in plasma LDL. Much of the information about the mechanism for this reduction comes from studies in cell culture and in experimental animals (Groot et al., 1992). Cultured human fibroblasts respond to an inhibition of HMG-CoA reductase by accumulating increased amounts of the enzyme (Brown et al., 1978). The increase is attributable to an increase in the rate of transcription of the HMG-CoA reductase gene, an increase in the rate of translation of the mRNA, and a decrease in the rate of degradation of the protein. Through these compensatory mechanisms, cultured cells can increase the amount of HMG-CoA reductase sufficiently to restore rates of cholesterol synthesis almost to normal, even in the presence of relatively high concentrations of the inhibitor. An increase in HMG-CoA reductase also occurs in the livers of rabbits, hamsters, and rats (Endo et al., 1979; Tanaka et al., 1982) treated with these inhibitors. A similar adaptation is presumed to occur in humans. Hamster has been reported to respond to hypocholesterolemic drugs (Suckling et al., 1991) and develop atherosclerosis under appropriate conditions (Nistor et al., 1987; Sima et al., 1990), although differences between the hamster and man, which hamper the use of this species as a model for human atherosclerosis, have been noted (Nikkari et al., 1991). Rats also respond to hypocholesterolemic drugs but in a different way to hamsters. Rats are very capable of modulating their hepatic cholesterol synthesis and this is the first response to drugs such as bile acid sequestrants and HMG-CoA reductase inhibitors. However, hyperlipidemia can be induced and in some cases this can lead to atherosclerosis (Russell et al., 1990;
Vance and Russell, 1990; Russell et al., 1991). Inhibition of mevalonate and cholesterol synthesis by inhibitors of HMG-CoA reductase such as mevastatin and lovastatin prevents farnesylation and blocks cells growth (Maltese and Robishaw, 1990). It has been postulated that inhibitors of HMG-CoA reductase such as lovastatin used in the treatment of hypercholesterolemia, may also be useful for cancer chemotherapy (Schafer et al., 1989). HMG-CoA reductase inhibitors and/or inhibitors of Ras farnesylation protein transferase could prevent farnesylation of Ras proteins, inhibiting the growth of Ras-dependent tumour cells (Goldstein and Brown, 1990). Lovastatin and sodium phenylacetate, an inhibitor of isopentenyl pyrophosphate synthesis is in clinical trials for the treatment of malignant gliomas (Shack et al., 1994).

One of the areas, which have attracted a great deal of attention, is antioxidant nutrition in the control of degenerative diseases such as atherosclerosis and cancer (Ames, 1983; Ong and Packer, 1992). Peroxidation of the cellular membrane lipids is a basic reaction, which results in the deterioration of unsaturated fatty acids in the membrane. This process has been implicated in several human diseases and in the toxicity of xenobiotics (Yagi, 1982; Halliwell and Gutteridge, 1989; Sies, 1991). If lipid peroxidation is triggered, it can inactivate cellular components and its products can have serious consequences on almost all the crucial molecules leading to diseased conditions (Halliwell and Gutteridge, 1989; Sies, 1991). The peroxidation products can also cause the formation of 8-hydroxydeoxyguanosine whose presence in the genetic material can lead to mutagenesis and carcinogenesis (Kuchino et al., 1987; Park and Floyd, 1992). The susceptibility of tissues to lipid peroxidation is influenced by the lipid and antioxidant composition of cellular membranes, which in turn may be controlled, by dietary composition (Clegg, 1973; Manorama and Rukmini, 1992).
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 (Kasperek, 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 (Kasperek, 1980a) (Fig. 1.8). Tocopherols predominate in certain oils such as corn oil, soyabean 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, b & c). The ability of barley extracts to lower 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
Fig. 1.8. Structures of tocotrienols and tocopherols
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, soyabean 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; Miller 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 (Parker et al., 1993). 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). These activities of tocotrienols in HepG2 cells mirror the actions of the putative nonsterol feedback regulators derived from mevalonate in cultured cells (Goldstein and Brown, 1990). In contrast to the effects of 25-hydroxycholesterol, T₃ does not suppress LDL receptor protein in HepG2 cell membranes as demonstrated by western blotting.

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. HDL₃-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 HDL₃-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, Ph.D. thesis, 1999; Minhajuddin et al., 1999). 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). Our 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, HDL2-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; Minhajuddin, PhD Thesis, 1999).

Based on strong hypocholesterolemic and antioxidant properties of tocotherienols, our laboratory has also investigated antitumour activity of tocotherienols 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 tumours 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 tumour growth (Iqbal. Ph.D. thesis. 1999).

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. M.D thesis, 2001; Baliarsingh et al., 2002).

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 spectroscopies 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, apoB, 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). 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). 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). 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).

Since the discovery of vitamin E, in Berkeley by H.M. Evans in 1992, when it was first described as antisterility agent, many scientists and physicians have sought to elucidate its biochemistry, health benefits and clinical applications (Lester, 1992). Vitamin E has been well accepted as nature’s most effective lipid soluble, chain-breaking antioxidant. It is the first line of defence against lipid peroxidation protecting polyunsaturated fatty acids in cell membranes through its free radical quenching activity in biomembranes at an early stage of free radical attack (Horwitt, 1986). After being oxidized in this process and before being decomposed, vitamin E can be re-reduced by ascorbic acid and glutathione. This reaction is dependent on the concentration of these substances and/or the enzymes that maintain them in their reduced form.
The antioxidant activity of T and T₃ is mainly due to their ability to donate their phenolic hydrogens to lipid free radicals (Burton and Ingold, 1986; Pokorny, 1987; Burton and Ingold, 1988; Burton and Traber, 1990). The mechanism for the inhibition of lipid peroxidation in biological membranes by T and T₃ can be outlined as follows: The peroxidation proceeds in 3 phases; initiation, propagation and termination (Burton and Traber, 1990). In the initiation phase a carbon centered lipid radical R* is produced from a polyunsaturated fatty acid RH. This R* reacts with molecular oxygen in the propagation phase to form ROO*, which reacts with other RH forming a hydroperoxide, ROOH.

\[
R^* + O_2 \rightarrow ROO^*
\]

\[
ROO^* + RH \rightarrow ROOH + R^*
\]

This propagation process continues and consumes the valuable polyunsaturated fatty acids. In the termination phase, the chain reaction stops when a peroxyl radical (ROO*) combines with another ROO*.

\[
ROO^* + ROO^* \rightarrow \text{Inactive product}
\]

However, tocotrienols (T₃-OH) and tocopherols (T-OH) can intercept the peroxyl radical more rapidly than can polyunsaturated fatty acids by the following reactions:

\[
T_3\text{-OH} + ROO^* \rightarrow T_3\text{-O}^* + ROOH
\]

\[
T_1\text{-O}^* + ROO^* \rightarrow \text{Inactive product}
\]
The $T_3-O^*$ radical is unable to continue the chain and reacts with the peroxyl to form inactive product.

Although, it is generally agreed that the relative antioxidant activity of $T$ in vivo is in the order of $\alpha>\beta>\gamma>\delta$ (Dillard et al., 1983; Burton and Ingold, 1986; Burton and Traber, 1990; Verlangieri and Bush 1992), there is a wide spread confusion concerning their relative potency in vitro (Burton and Ingold, 1981). The chemical structures of $T$ and $T_3$ support hydrogen donating power in the order of $\alpha>\beta>\gamma>\delta$ (Pokorny, 1987). This order also was obtained when the activity of the four $T$ was compared in a homogenous solution in dichlorobenzene (Burton and Ingold, 1981), but a reverse order ($\delta>\gamma>\beta>\alpha$) was obtained when the relative antioxidant properties were compared in fats, oils and lipoproteins in vitro (Lea and Ward, 1959; Olcott and Van Der Ven, 1968; Parkhurst et al., 1968; Koskas et al., 1984; Esterbauer et al., 1989; Gottstein and Grosch, 1990). The reason behind this order is not yet clearly understood. Several studies have demonstrated the ability of tocopherols to prevent ex vivo and in vitro LDL-C oxidation and significantly reduce the development of atherosclerotic lesions (Carew et al., 1987; Esterbauer et al., 1991; Mao et al., 1991), risk of coronary heart disease (CHD) (Rimm et al., 1993; Stampfer et al., 1993) and ischemic heart disease (Gey, 1995). Even though, $\alpha-T$ has been labeled as the most efficient chain breaking antioxidant. tocotrienols are known to be more potent antioxidant than tocopherols (Suarna et al., 1993; Kamat and Devasagayan, 1995; Kamat et al., 1997). Tocotrienols have been shown to have greater free radical scavenging properties as cell membrane constituents than $T$ (Yamaoka and Carrillo, 1990; Serbinova et al., 1991). Recently, it was shown that compared to $\alpha-T$, $\alpha-T_3$ possesses a 40-60 times higher antioxidant activity against $Fe^{2+}$ + ascorbate- and $Fe^{2+} + NADPH$-induced
l lipid peroxidation in rat liver microsomal membranes and 6.5 times greater protection of cytochrome P-450 against oxidative damage (Serbinova et al. 1991). This higher antioxidant potency of α-T₃ as compared to α-T is attributed to the combined effects of three properties; its higher recycling efficiency from chromanoxyl radical, its more uniform distribution in membrane bilayer, and its stronger disordering of membrane lipids which makes interaction of chromanols with lipid radicals more efficient.

Several animal studies have been conducted to test the hypothesis that antioxidants delay atherosclerosis. Wojcicki reported a statistically significant 25 per cent reduction in aortic atherosclerotic lesions in hypercholesterolemic mongrel rabbits fed 10 mg/kg/day of vitamin E compared to controls (Wojcicki et al., 1991) and Verlangieri reported a 54 per cent lesion reduction in monkeys fed 108 IU of vitamin E per day compared to controls (Verlangieri and Bush, 1992). Qureshi et al. (1991a & b) have reported a reduction in both total and LDL-cholesterol in human hyperlipidemias and pigs supplemented with T₃. Supplementation of γ-T₃ along with an atherogenic diet to rats for six weeks has been shown to lead to decreased plasma lipid and lipoprotein concentrations. In addition, a decrease in the plasma lipid peroxidation was shown (Watkins et al., 1993). Similar observations were reported in hypercholesterolemic rabbits by Teoh et al. (1994). Wahlquist et al. (1992), however, observed a differential response to T and T₃ supplementation in human subjects without any change in serum lipids. Tomeo et al. (1995) also reported that T₃ significantly decreased plasma lipid peroxidation in patients with hyperlipidemia and carotid stenosis with no change in their lipid and lipoprotein parameters. Similarly, Mensink et al. (1999) recently reported that T₃ had no markedly favourable effects on the serum lipoprotein profile in men with slightly elevated lipid concentrations. Experiments involving the effects of T₃ on apoB (LDL) and apoA-I (HDL) and apoB/apoA-I ratio has
been reported in chickens, swine and humans. The apoB/apoA-1 ratio, considered being a better indicator than LDL/HDL ratio, for assessment of CHD, was reduced in T3 treated subjects (Qureshi et al., 1991a&b). Recently, the mechanism of action of T3 on apoB metabolism in HepG2 cells has been investigated (Wang et al., 1998; Theriault et al., 1999b).

For a long time now it has been shown that dietary regimen in conjunction with exercise favourably alter the lipid parameters in hypercholesterolemic individuals. Based on the current knowledge dietary recommendations to optimize plasma lipid profiles and thus reduce the risk of coronary heart disease are currently focused on the total fat content, the fatty acid profile, and the cholesterol content of the diet (Dietary Guidelines, 1988). Public health programmes for prevention of CAD in developed countries recommend changes in dietary habit and patterns of various components of diets, especially dietary fat. Recently a reduction in dietary intake of total fat, particularly animal fat with a limited amount of saturated fatty acid and polyunsaturated fatty acid, has been suggested (Lesserre et al., 1985). The American Heart Association (1982) recommends that total fat content of the diet should not exceed 30% of total calories, saturated fatty acid should not exceed 10%, monounsaturated fatty acid should not exceed 10% and polyunsaturated fatty acid should not exceed 10% of calories. Lesserre et al. (1985) reported that linolenic acid (18:3) should provide 0.5-1.0% of total calories.

Polyunsaturated fatty acids are abundantly available in vegetable oils like safflower oil, sunflower oil and corn oil which were found to lower blood total and LDL-cholesterol levels, as revealed by epidemiological studies (Berkson and Stamler, 1981). A number of studies in humans and animals have shown that RBO is as effective as other vegetable oils in lowering plasma cholesterol levels (Rukmini and Raghuram, 1991; Lichtenstein et al., 1994). In some cases, RBO lowered plasma cholesterol more effectively than other commonly used vegetable
oils rich in linoleic acid (Rukmini and Raghuram, 1991); this effect was attributed to the presence of T and T$_3$ in the RBO (Nicolosi et al., 1991). However, Qureshi et al. (1991a,b), confirmed the impact of tocotrienols on cholesterol, specifically the LDL-cholesterol, in animal and human studies. The tocopherols on the other hand do not lower serum cholesterol (Qureshi et al., 1989). Recently, biological properties of tocotrienols such as hypolipidemic, antioxidant and antitumour, have been reviewed in detail (Theriault et al., 1999a).
1.1 Objectives of the present investigation

Based on the above discussion, in the first part of the thesis we have investigated the hypolipidemic, antioxidant and antiatherosclerotic impacts of tocotrienol rich fraction. TRF (isolated from purified edible grade RBO), and a commercial preparation of purified tocotrienol rich fraction. Tocomin (isolated from palm oil), when fed together with 0.33% cholesterol rich diet to rabbits for 22.4 weeks and compared with hyperlipidemic control. The efficacy of 16.2 mg% TRF and 6.97 mg% Tocomin in preventing the increase in plasma TG, TC, VLDL-C, LDL-C, HDL-C, and lipid peroxide levels in hyperlipidemic rabbits was investigated. In addition, we have investigated the impact of TRF and Tocomin on ex vivo and in vitro LDL oxidation, and on the formation of fatty streak lesions in the aortas. The second part of the thesis deals with the investigations related to the role of dietary 0.33% nonoxidized cholesterol or the same diet containing 0.33% cholesterol of which 5% was oxidized, in experimental hyperlipidemia; plasma and liver lipid peroxidases; ex vivo and in vitro oxidative modification of LDL. In addition, this part also deals with the hypolipidemic, antioxidant and antiatherosclerotic impacts of dietary tocotrienols (TRF) fed to rabbits for 10 weeks together with a diet containing 0.33% nonoxidized cholesterol or the same diet containing 0.33% cholesterol of which 5% was oxidized. Effect of feeding 50 mg% TRF (25 mg% TRF plus 25 mg% Tocomin), mixed with either nonoxidized cholesterol rich diet or a 95% nonoxidized and 5% oxidized cholesterol rich diet on liver TC, plasma TG, TC including FC and EC, VLDL-C, LDL-C, HDL-C, HDL₃-C and HDL₂-C, and plasma as well as liver lipid peroxidases was investigated. Moreover, antioxidant impacts of TRF on baseline levels of ex vivo diene conjugation of LDL, rates of conjugated diene formation in LDL, TBARS contents of LDL, and lag phase time of copper-induced oxidation in LDL, isolated from plasma of rabbits in each group was undertaken. We have also examined the role of oxidized...
cholesterol in the acceleration of fatty streak lesions in the aorta in comparison to rabbits fed only nonoxidized cholesterol. Similarly, efficacy of tocotrienols-mediated protection against the formation of early atherosclerotic lesions in aortas of hyperlipidemic rabbits in both groups was also investigated.