REVIEW OF LITERATURE

Cardiovascular disorders like atherosclerosis are responsible for large number of deaths in the western world and presently these disorders are also of concern in developing nations. India being a developing country with industrialization and modernization on an increase, cardiovascular disorders are going to be the biggest problem of the coming time. Atherosclerosis is a disease of multiple casualty. In fact, it is certainly not a single disease but more likely a group of different diseases. Similar end result, atheroma, may stem from more than one initiating factors and be reached via different but probably interacting pathways (Steinberg, 1989). Hypercholesterolemia represents one of the very important and recognized risk factor for atherosclerosis (Descamps et al., 2003). High fat diet feeding leads to cholesterol deposition in the arterial wall (Brown and Goldstein, 1984). Feeding of cholesterol as a means of establishing experimental atherosclerosis dates back to the work of Anitschkow and Chalatow (1913) and is being followed presently also. Strong association between coronary risk and lipoproteins concentration in plasma has been well documented in several epidemiological studies (Castelli et al., 1977).

Compelling evidences indicate the importance of the types of fat than total amount of fat with respect to the risk of cardiovascular disorders (Seidel et al., 2005). The seminal metabolic studies conducted by Hegested et al. (1965) and Keys and Parlin (1966) have established that the type of fat but not total amount of fat predicts serum cholesterol levels. Consistent with the metabolic studies, epidemiological studies strongly support the idea that types of fat are more important than total amount of fat in determining the risk of cardiovascular disorders. Controlled clinical trials have also shown that replacing saturated fat with polyunsaturated fat is more effective in lowering serum cholesterol and reducing risk of cardiovascular disorders than simply reducing total fat consumption (Hu et al., 2001).
Atherosclerosis has been thought to be the response of vascular wall to injury (Ross and Glomset, 1976; Ross, 1986) having many of the features of inflammatory response in other tissues (Joris et al., 1983; Ross, 1986). It is broadly accepted now that atherosclerosis is a complex disorder involving several types of cells, especially the macrophages, endothelial cell and arterial smooth muscle cells as well as an interaction of two main processes, lipid (mainly cholesterol and its esters) accumulation and cell proliferation. The evidence in support of this comes from the work of Orekhov et al., (1990) which indicates that the major event inducing atherogenesis is primary cellular lipidosis followed consequently by stimulation of cell proliferation. A growing body of evidence now strongly suggests the role of T-cells in the initiation of atherosclerosis (Emeson and Robertson, 1988; Hansson et al., 1989; Van der Wal et al., 1989; Capron, 1993; Drew and Tipping, 1995; Stary et al., 1995).

Various researchers have given different theories for the pathogenesis of atherosclerosis. Badimon et al., (1993) have suggested the following sequence of events: (1) vascular injury, (2) monocyte recruitment, macrophages formation and lysis, (3) lipid deposition, (4) platelet growth factors. (5) role of vascular smooth muscle cells. (6) synthesis of extracellular matrix. Hypercholesterolemia has been observed to play most important role at every sequence of events.

**Hypercholesterolemia and Cardiovascular Disorders**

At the dawn of the 21st century, we consider it both appropriate and timely to review the cholesterol story of the past century. The French chemist, M E Chevreul is credited with the discovery of cholesterol in 1815. Although much of the research on cholesterol in the past century has been driven by curiosity, the link between cholesterol and human disease has also been a major driving force. Cholesterol, the most common member of the steroid family, is an essential constituent of the cell membrane and a precursor of steroid hormones and bile acids. Liver (hepatocytes) is the major site for cholesterol biosynthesis in vertebrates, though all the cells in body are capable of its synthesis. In contrast the
catabolism of cholesterol is very limited in most cells and the major organs for cholesterol catabolism are liver steroidogenic cells, in which cholesterol is converted to bile acids or steroid hormones. Due to this reason most of the cholesterol molecules in the other tissues must be removed and transported to either liver or steroidogenic cells. A large amount of cholesterol in the body is produced by hepatocytes and supplied to other cells. Intracellular and extracellular cholesterol levels are tightly maintained within a narrow range by an intricate transcriptional control mechanism. Extra cellular cholesterol transport is largely mediated by plasma lipoproteins. The delivery of cholesterol to the liver is mainly carried out by LDL (Brown et al., 1986) and other apoB containing lipoproteins via the receptor mediated-endocytosis of the lipoproteins and perhaps in minor part by the high density lipoprotein (HDL) via the selective uptake of its cholesteryl esters in certain organs (Acton et al., 1996).

Defects in the cholesterol metabolism are the major cause of cardiovascular disease and hundreds of papers have been published on the link between cholesterol metabolism and cardiovascular disease (Pasha, 2005). Patients with familial hypercholesterolemia (FH) have a defect in the functioning of the LDL receptor. These patients are therefore unable to take up cholesterol from LDL in the plasma efficiently and cholesterol biosynthesis is not decreased. The consequence is a large increase in the plasma cholesterol, which leads to premature atherosclerosis (Eckardstein et al., 2001). The concept that dietary cholesterol contributes to hypercholesterolemia and coronary heart disease (CHD) risk has been a fundamental part of public health policy and dietary recommendations. In 1970s, the recommendation that cholesterol be restricted in the diets of the general population, and severely restricted in the diets of those with hypercholesterolemia, was based on three lines of evidences: (1) animal studies showing that dietary cholesterol induces hypercholesterolemia and atherogenesis in some species; (2) epidemiological surveys reporting a positive relationship between dietary cholesterol and CHD incidence; and (3) clinical
observations that feeding cholesterol increases plasma total cholesterol levels. Based on these evidences, a number of organizations recommended restricted dietary cholesterol levels for the population in an effort to reduce the plasma cholesterol levels and CHD risk (Krauss et al., 1996).

Anitschkow (1913) first documented the important role of dietary cholesterol in the pathogenesis of atherosclerosis by using a rabbit model. Since that time, a myriad of studies have shown that raising dietary cholesterol resulted in higher plasma cholesterol concentrations (Connor et al., 1964; Quintao et al., 1971; Brown et al., 1991; Hopkins, 1992) and is associated with increased risk of CHD (Armstrong et al., 1975; Kromhout et al., 1985; Kushi et al., 1985; Pasha, 2005). Excess of cholesterol in the serum derived from endogenous synthesis/dietary sources initiates atherosclerosis by accumulation of lipids in the cells of arterial wall and provoking atheroma growth. Strong et al., (1978) have studied the relationship between the incidence of coronary intimal atherosclerotic lesions and age at the onset of hypercholesterolemia in autopsied cases. According to their report the incidence increased with age, often beginning at 30 years of age and reaching a peak at 50 to 60 years. However, the incidence of so-called fatty streaks i.e. early atherosclerotic lesions, increased from 10 years of age, reaching a peak by 30 years. Risk factors such as hypercholesterolemia apparently play an important role in the development of early atherosclerotic lesions. Familial Hypercholesterolemia (FH), patients are believed to be at increased risk of developing atherosclerosis due to exposure to high cholesterol levels from an early age (Dedoussis et al., 2004).

Wissler and Vesselinovitch (1983) used an atherogenic diet to produce aortic atherosclerotic lesions in the monkeys and observed that the cholesterol lowering agents like cholestyramine, probucol produced a marked regression of atheromatous lesions. Kita et al., (1987) have reported that the administration of probucol to Watanabe heritable hyperlipidemic (WHHL) rabbits prevented the progression of atheromatous lesions of the aorta. Kobayashi et al., (1989) and
Fukuo et al., (1991) have demonstrated that an inhibitor of 3-hydroxy-3-methyl glutaryl coenzyme A reductase (rate limiting enzyme in the cholesterol biosynthesis) has been reported to be effective in preventing the progression of aortic lesions in Watanabe heritable hyperlipidemic rabbits with hyperlipidemia loads. Tomochika et al., (1996) have shown that administration of probucol and the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor provastatin to the patients with heterozygous Familial Hypercholesterolemia resulted in a significant reduction of cholesterol level associated with a significant morphological and physiological regression of atherosclerotic lesions.

Best et al., (1999) and Rodriguez et al., (2002) have demonstrated that diet-induced hypercholesterolemia has been associated with coronary artery endothelial dysfunction and impairment in endothelium-dependent coronary vasorelaxation, both at the epicardial and the microvascular level, endothelial dysfunction is limited to the pertussis toxin–sensitive Gi-protein– dependent pathway leading to nitric oxide formation (Shimokawa and Vanhoutte, 1989). NO is known to react with superoxide anion (O$_2^-$) to give peroxynitrite (ONOO$^-$) which is a highly reactive free radical species (White et al., 1994). Rubanyi and Vanhoutte (1986) have demonstrated that hypercholesterolemia increases the production of the superoxide anion, which scavenges the endothelium-derived vasodilator, nitric oxide. Atherosclerosis is associated with impairment of endothelium dependent vasorelaxation in animal models (Jayakody et al., 1985).

It has been observed in literature that people having cholesterol levels< 200 have suffered from myocardial infarction, also there are patients with heterozygous familial hypercholesterolemia and cholesterol levels>300 who somehow survive into their 70s with no clinically evident CHD. So this suggests clearly that in addition to total cholesterol level there must be some other factors also that modulate the impact of hypercholesterolemia on the blood vessel wall, increasing or decreasing the pace at which atherosclerosis progresses. So, in other words we will have to move beyond cholesterol also.
Low Density Lipoprotein (LDL) and Cardiovascular Disorders

The statement by Noble Award winners Brown and Goldstein (1984) that “more the LDL in the blood more rapidly atherosclerosis develops” has dominated research on atherosclerosis since then.

Low density lipoprotein (LDL) is a large spherical particle, 22–29 nm in diameter, composed of a core of esterified cholesterol and triglyceride, a surface lipid coat of unesterified cholesterol and phospholipid, an essential structural protein, apolipoprotein B (apoB), that modulates LDL metabolism (Schumaker et al., 1994). Each LDL particle has one apoB molecule, which is recognized by LDL receptors that removes LDL from plasma. LDL is the major carrier of cholesterol in the blood and is most significantly associated with atherosclerotic plaque formation (Auwerx et al., 1989). LDL has long been implicated in the development of atherosclerosis. The most convincing evidence that these lipoproteins are causative factors in this disease is the genetic disorder. Familial Hypercholesterolemia (FH), in which homozygous patients develop massive LDL concentrations and frequently die within the second decade of life from complications of coronary artery atherosclerosis (Fredrickson et al., 1972). Several epidemiological studies have suggested that the high concentration of LDL cholesterol in plasma has been shown to be highly correlated to the incidence of coronary heart disease (Castelli et al., 1990).

An increase in the plasma LDL levels lead to its accumulation in the intima, where it is modified. This modified LDL activates the endothelial cells, lining the vessel wall, attracting monocytes from the circulation, which subsequently will adhere to the endothelial cells, cross the endothelial layer to enter the media, differentiate into macrophages, and eventually become foam cells. These foam cells are characterized by a massive accumulation of cholesterol esters resulting from the unrestricted uptake of oxLDL (Ross et al., 1993).

Kita et al., (1987) in their studies have concluded that oxidative modifications of low-density lipoprotein (LDL) by free radicals play a major role
in initiation of atherosclerotic lesions. Oxidized LDL has atherogenic actions in the vascular wall including activation of inflammation. Circulating oxidized LDL is associated with increased risk of coronary heart disease (Ehara et al., 2001; Holvoet et al., 2001; Stocker and Keaney, 2004). Henrikson et al., (1981) have demonstrated that oxLDL has the ability to induce cholesterol accumulation in macrophages and this basically was the fact behind the hypothesis that oxidation of LDL might be an important step in the atherogenic process. Over the following years a number of additional properties of oxLDL were described that could in principle contribute to its atherogenicity. For example, oxLDL is itself a chemotactic agent for monocytes (Quinn et al., 1987) and for T cells (McMurray et al., 1993) but not for B cells. This is consistent with the fact that lesions contain primarily monocytes and T cells. Hessler et al., (1983) stated that the cytotoxicity of LDL for cultured endothelial cells was due to its oxidative modification and that this clearly could be atherogenic. Studies in Fogelman's laboratory showed that oxLDL could stimulate the release of macrophage colony-stimulating factor (M-CSF) (Rajavashisth et al., 1990) and of monocyte chemoattractant protein-1 (MCP-1) (Cushing et al., 1990) from endothelial cells, which would facilitate the development of fatty streak lesions by recruiting monocytes and facilitating their differentiation into tissue macrophages.

Early studies of LDL metabolism in patients with Familial Hypercholesterolemia revealed that in addition to the LDL clearance defect, they overproduce LDL (Packard et al., 1976; Soutar et al., 1977) and small VLDL particles (James et al., 1989). VLDL is the metabolic precursor of LDL and is converted to LDL through the action of lipoprotein lipase, a triacylglycerol lipase that acts upon VLDL while it circulates in the bloodstream (Havel and Kane, 1995). Increased production of VLDL can lead to increased LDL simply by providing more precursors. In addition, impaired clearance of VLDL remnants can lead to LDL overproduction (Bilheimer et al., 1982).
LDL particle size is the major determinant of the extent of atherogenecity caused by LDL. Both large and small LDL compared with intermediate size LDL have reduced affinity for the LDL receptor (Nigon et al., 1991; Campos et al., 1996; Berneis et al., 2005). Decreased clearance of these forms of LDL by the liver and steroidogenic tissues suggests the increased uptake of this type of LDL by the arterial wall. Campos et al., (1997) have demonstrated that small LDL remains in the plasma for longer time than large LDL. This may be caused by reduced exposure on small LDL of the region of apoB that binds to the LDL receptor, an interaction that is necessary to clear LDL from the circulation. The long residence time in plasma for small LDL could foster atherosclerosis, also, small LDL can enter the arterial intima more readily than other LDL (Nordestgard, 1989). But, in an in-vivo study of transvascular transport of LDL, Kornerup et al., (2002) in humans have found that there was no correlation between atherogenesis and LDL particle size, their findings suggested that for every unit of time, large LDL is just as likely as small LDL to enter the arterial intima. Because large LDL has more cholesterol ester than small LDL, a large LDL particle would deposit more cholesterol into plaque than small LDL. Small LDL binds to arterial proteoglycans (Camejo et al., 1990) in the arterial wall, but so does large cholesterol-rich LDL (Manning et al., 1994). Proteoglycans exist on the endothelial cell surface as well as inside the intima. Proteoglycans after binding on endothelium may facilitate lipoprotein entry into the vascular intima and activate or accelerate the plaque progression.

However, small LDL size is substantially correlated with high triglycerides and low HDL, and mildly related to obesity and perhaps insulin resistance (McNamara et al., 1987; Austin et al., 1990; Reaven et al., 1993). These metabolic connections complicate efforts to determine whether small LDL particles have an especially strong relationship to coronary heart disease.
Low Density Lipoprotein Receptor (LDL-R)

LDL receptors were discovered by Brown and Goldstein in 1973. The LDL-R is a highly conserved integral membrane glycoprotein consisting of five domains after cleavage of signal sequence (Schneider, 1989). In order of appearance from the aminoterminus, these domains are (1) the ligand binding domain; (2) a domain that has a high degree of homology with precursor to the epidermal growth factor (EGF); (3) a domain that contains a cluster of O-linked carbohydrate chains; (4) a transmembrane domain; and (5) a short cytoplasmic region (Schneider, 1989). It is the ligand-binding domain that mediates the interaction between LDL-R and lipoproteins containing apolipoprotein B or apolipoprotein E (Esser et al., 1988). Molecular weight of mature LDL-R is apparently 160kD (Tolleshaug et al., 1982). LDL-R locus spans about 45 kb of DNA and is localized on the distal short arm of chromosome 19 (Lindgren et al., 1985). The gene is made up of 18 exons, which are separated by 17 introns (Schneider, 1989).

The LDL receptor plays a critical role in the regulation of plasma LDL levels by mediating approximately two thirds of LDL clearance (Langer et al., 1972; Bilheimer et al., 1979; Lieu et al., 2003). The LDL receptor binds to cholesterol rich LDL and removes it from plasma and thereby regulates the plasma cholesterol level (Brown and Goldstein, 1986). The liver is a tissue that processes a lot of cholesterol daily and it is the organ that demonstrates the highest level of LDL-R activity (Rudel et al., 1986). The LDL receptor mediates the removal of LDL and remnant lipoproteins from circulation by binding to apolipoprotein B-100 (apoB-100) and apolipoprotein E (apoE). Loss of LDL receptor function leads to decreased LDL catabolism and elevated LDL levels (Brown and Goldstein, 1986).

LDL receptor levels are affected by diet, hormones, and most dramatically by mutations in the LDL receptor locus that leads to Familial Hypercholesterolemia (FH). It was recognized that mutations affecting the
function of LDL-R are responsible for Familial Hypercholesterolemia (Hobbs et al., 1992; Kaiser et al., 2002; Dedoussis et al., 2004). Intensive studies at molecular level have identified many mutant alleles at the LDL-R locus (Schneider, 1989). The individuals who have inherited two mutant alleles (FH homozygotes) are more severely affected than those with one mutant allele (FH heterozygotes) (Schneider, 1989). The presence of one mutant allele is found in 1 in 500 persons, while one individual among about one million carries two mutant genes at the LDL-R locus. FH is the outstanding example of a single gene mutation that results in obligatory atherosclerosis. Myocardial Infarction, angina pectoris and sudden death occur usually before the age of 15 in these individuals. Another much discussed example of gene mutation in literature is WHHL (Watanabe Heritable Hyperlipidemic) rabbits discovered in 1978 by Yoshio Watanabe at Kobe University. They are homozygous for a mutant LDL-R gene and produce less than 5% of the normal number of LDL-R. They have high circulating LDL from the time of birth and develop atherosclerosis leading to heart attacks by the age of two. Kita et al., (1982) have demonstrated in WHHL rabbits that as in these rabbits liver cells lack LDL-R, so more IDL particles along with LDL remained in circulation and these IDL particles were eventually converted into more than normal amount of LDL so they concluded reduction in the LDL-R has two effects in these rabbits- increased production and decreased removal of LDL, that act synergistically to raise the LDL level.

Brown and Goldstein (1984) have demonstrated that much of atherogenecity in general population is caused by a dangerously high blood levels of LDL resulting from failure to produce enough LDL-R. Wiseman et al., (1993) showed that the magnitude of the hypercholesterolemia is associated with the variation in the LDL-R gene. Liu et al., (1997) demonstrated that on feeding high fat diet, the liver LDL-R mRNA expression was lower in mice in comparison to control group. Gene manipulation has produced significant effects on LDL receptor expression (Ouguerram et al., 2004). Yokode et al., (1990) have achieved
over expression of LDL-R by injecting mouse eggs with cDNA encoding the human LDL-R. Mice expressing this transgene had high levels of LDL-R in liver. In these mice the hepatic uptake of LDL increased. When these transgenic animals were given low fat diet, the plasma apoB or apoE levels were virtually undetectable. When maintained at a normal laboratory diet, serum cholesterol level was found to be less than 50% of that in their normal siblings. On high cholesterol diet feeding, the cholesterol level was not significantly increased.

Ishibashi et al., (1993) have demonstrated that, the mice that lack functional LDL-R as a result of gene knock out accumulate significant amount of LDL cholesterol in their plasma. They have also suggested that when LDL-R were absent, mice became hyper responsive to dietary cholesterol and these mice were found to be highly susceptible to the formation of atherosclerotic vascular lesions when fed with a cholesterol rich diet.

Herz and Gerard (1993) developed a recombinant replication-defective adenovirus vector containing an expressible cDNA copy of human LDL-R driven by cytomegalovirus (CMV) promoter. This virus elicited the expression of high level of human LDL-R in more than 90% of mouse hepatocytes, and this enhanced markedly the uptake of LDL by the liver.

**Thyroid Hormone (T3/T4) Status and Cardiovascular Disorders**

Thyroid hormones and lipids share a long history because the link between thyroid disease and lipid disorders was described more than 70 years ago. Thyroid hormones have relevant effects on the cardiovascular system (Klein and Ojamaa, 2001). Many symptoms and signs recognized in patients with overt hyperthyroidism and hypothyroidism are due to the increased or reduced action of thyroid hormones on the heart as well as vascular system and the related hemodynamic derangements. In recent decades, it has emerged that subclinical thyroid dysfunction may affect the cardiovascular system, which may increase cardiovascular risk. It is becoming increasingly apparent that acute and chronic
cardiovascular disease may alter thyroid hormone metabolism and contribute to cardiovascular impairment.

There are a growing number of human cardiac disease states in which thyroid hormone metabolism is altered leading to a decrease in serum T₃ level. Within 48 hrs after acute myocardial infarction (Franklyn et al., 1984) or within 6–24 hrs after cardiac surgery requiring cardiopulmonary bypass in adults and children (Mainwaring et al., 1994; Klemperer et al., 1995), serum T₃ level was found to be decreased. In children, the fall in T₃ was more pronounced and prolonged in patients with a more complex surgical procedures (Mainwaring et al., 1994). T₃ replacement therapy to restore serum T₃ levels to normal improved the postoperative outcome and cardiac function in newborn children without untoward effects (Chowdhury et al., 1999; Bettendorf et al., 2000). In patients with congestive heart failure, it has been observed that as many as 30% have low T₃ levels that correlate with the severity of the clinical assessment of heart failure (Hamilton et al., 1998).

Studies have also shown that decrease in thyroid hormone levels in body causes qualitative changes in circulating lipoproteins that increase their atherogenicity. Sundaram et al., (1997) have shown that LDL is more susceptible to oxidation in patients with hypothyroidism. Increased levels of lipoprotein (a) [Lp(a)], a particularly atherogenic LDL variant in which apolipoprotein(a) and apoB are covalently bound, have also been reported in hypothyroidism, compared with euthyroid controls. Several studies have shown decrease in the Lp(a) concentration after T₄ treatment of hypothyroid patients (De Bruin et al., 1993. Triguero. 1998). Mamiya et al., (1989) and Masaki et al., (1992) have shown that thyroid hormones can inhibit the collagen induced platelet aggregation and can directly relax vascular smooth muscles (Ishikawa et al., 1989). The effect of thyroid hormone level on the coagulation and anticoagulation system, which plays a prominent role in atherosclerosis and its complications, could be of even more important as hypothyroidism is accompanied by hyper-coaguable state (Marongiu
et al., 1992). Further enhanced T₃ and T₄ levels (hyperthyroidism) increase the levels of thrombomodulin, an endothelial cell surface protein that binds to thrombin and neutralizes its clotting activity (Morikawa et al., 1993).

Despite various assessments of the literature, the relationship between mild thyroid failure and serum lipid levels is still ambiguous (Toft, 1994; Tanis et al., 1996; Cooper, 1998; Helfand and Redfern, 1998). Some cross-sectional studies suggest that serum cholesterol levels are significantly higher in individuals with mild thyroid failure than in euthyroid individuals (Althaus et al., 1988; Kung et al., 1995; Muller et al., 1995; Bauer et al., 1998). In other cross-sectional studies, the differences are not statistically significant (Tunbridge, 1977; Geul et al., 1993; Parle et al., 1992). Still, other cross-sectional studies of hypercholesterolemic individuals report higher prevalence of mild or overt thyroid failure than in normocholesterolemic control groups (Ball et al., 1991; Oetten et al., 1994).

**Hypothyroidism and Cardiovascular Disorders**

Several cohort studies have suggested a strong link between hypothyroidism and various indicators of atherosclerotic disease (Jung et al., 2003). The first case-control study by Vanhaelst et al., (1967) compared autopsy findings in 25 patients with myxedema with 50 age-matched controls and found a greater prevalence and severity of coronary atherosclerosis in the hypothyroid group. In a subsequent case-control study performed by Steinberg (1968), women with myxedema had more severe coronary artery disease on autopsy than did age-matched women without myxedema. Gasper (1968) in another autopsy study took the converse approach by examining the thyroid glands of 55 patients who had died of atherosclerotic disease. All of the thyroids were found to have some abnormality, in size or cellular structure, compared with no abnormalities in four controls without atherosclerosis. A study of patients undergoing coronary angiography demonstrated that those who had inadequate therapy for hypothyroidism were more likely to have angiographic progression of coronary artery disease than those with adequate replacement (Perk and O’Neill, 1997).
Frank et al., (2004) have concluded that total cholesterol, VLDL and triglycerides level was significantly higher during hypothyroidism in adult mares in comparison to euthyroid subjects. Danese et al., (2000) and Siegmund et al., (2004) have observed that thyroid hormone replacement in patients with subclinical hypothyroidism can reduce abnormalities in cholesterol metabolism. Morris et al., (2001) have reported that lipid abnormalities are more common in patients with overt hypothyroidism and are thought to contribute to the disproportionate increase in cardiovascular risk in these persons. Vierhapper et al., (2000) have shown that the patients with subclinical hypothyroidism have elevated cholesterol levels. Manolio et al., (1992) in a population-based study have compared lipid levels in subclinical hypothyroid and euthyroid persons both were suffering from hypercholesterolemia.

Franklyn (1995) and Meier (2001) in their independent studies have strengthened the existing facts that thyroid replacement in patients with subclinical hypothyroidism can reduce lipid metabolism abnormalities. Canaris et al., (2000) and Pirich et al., (2000) in their studies of patients with subclinical hypothyroidism have shown that patients were very much prone to induce hypercholesterolemia. Hueston and Pearson (2004) have observed elevated average cholesterol and triglycerides levels in patients who have subclinical hypothyroidism and further demonstrated that, respondents with mild elevations in their serum TSH and normal T₄ levels were more likely to have elevations in cholesterol levels than respondents who had normal TSH levels. Michalopoulou et al., (1998) have investigated that almost 10% of the asymptomatic hypercholesterolemic patients have sub clinical hypothyroidism. Also prior to the availability of serum thyroid hormone measurements, serum cholesterol concentration was used to assist in the diagnosis of hypothyroidism (Bloomer and Kyle, 1959).

**Hypothyroidism and Traditional Cardiovascular Risk Factors**

The cardiovascular system responds to multiple endocrine signals and there are strong parallels between the mechanisms of endocrine and other types of
signals that influence the function of the cardiovascular system. There is substantial evidence that overt hypothyroidism alters several of the traditional risk factors for cardiovascular diseases. These studies support a plausible role of hypothyroidism in increasing the risk of atherosclerotic cardiovascular diseases, via increases in circulating levels of highly atherogenic low-density lipoprotein (LDL) cholesterol particles, induction of diastolic hypertension, altered coagulability, and direct effects on vascular smooth muscles. Furthermore, some evidence suggests that hypothyroidism may exacerbate the cardiovascular risks associated with cigarette smoking and insulin resistance (Cappola and Ladenson, 2003). Elevated levels of total cholesterol, LDL cholesterol, and apolipoprotein B are well-documented features of overt hypothyroidism (Staub et al., 1992).

The inverse relationships between atherosclerotic risk and concentrations of HDL cholesterol and its constituent apoprotein A1 are well known. Some studies have shown that hypothyroidism is associated with a lower HDL cholesterol level. In a report comparing 52 patients with sub clinical hypothyroidism and 18 with overt hypothyroidism with 46 euthyroid controls matched for age, sex, and body mass index, Althaus et al. (1988) found a significantly lower HDL cholesterol fraction in the sub clinically hypothyroid patients. Caron et al. (1990) reported that the HDL cholesterol level was significantly decreased among 29 women who had sub clinical hypothyroidism, compared with 41 euthyroid women matched for age and metabolic parameters. Furthermore, they also observed a significant increase in the HDL cholesterol level with T4 therapy.

Additional potentially atherogenic effects of hypothyroidism on lipid metabolism include, a reversible reduction in clearance of chylomicron remnants (Weintraub et al., 1999), reduced activity of cholesteryl ester transfer protein which is involved in reverse cholesterol transport pathway (Ritter et al., 1996; Tan et al., 1998) and decreased activity of hepatic lipase and lipoprotein lipase (Lam et al., 1986).
Hyperthyroidism and Cardiovascular Disorder

In patients with hyperthyroidism, cardiac output is 50 to 300 percent higher than in normal subjects. Patients with hyperthyroidism have increased left ventricular systolic and diastolic contractile function, a finding consistent with changes in the expression of contractile and calcium-regulatory proteins (Kiss et al., 1994; Ojamaa et al., 2002). The rate of increase in intraventricular pressure during systole, the left ventricular ejection fraction and the rate of blood flow across the aortic valve are all increased (Feldman et al., 1986). Sinus tachycardia is the most common rhythm disturbance in patients with hyperthyroidism. A large study found that less than 1% of cases of new-onset atrial fibrillation were caused by overt hyperthyroidism (Krahn et al., 1996). However, Forfar et al., (1979) have demonstrated that 13% of patients with unexplained atrial fibrillation have biochemical evidence of hyperthyroidism.

Treatment of hyperthyroidism is frequently associated with reversion to sinus rhythm; in one study, this occurred in 62 percent of 163 patients within 8 to 10 weeks after they returned to a euthyroid state (Nakazawa et al., 1982). In older patients with or without underlying heart disease or atrial fibrillation of longer duration, the rate of reversion to sinus rhythm is lower (Sandler and Wilson, 1959; Nakazawa et al., 1982; Nordyke et al., 1988; Klein and Ojamaa, 1998).

Patients with hyperthyroidism may occasionally have signs of heart failure (Klein, 1990; Polikar et al., 1993). In view of the increased cardiac contractile function of patients with hyperthyroidism (Mintz et al., 1991; Klein and Ojamaa, 1998), the development of heart failure is unexpected and raises the question of hyperthyroid cardiomyopathy (Forfar et al., 1982). As noted above, in most patients with hyperthyroidism, cardiac output is high, and the subnormal response to exercise (Forfar et al., 1982) may be the result of an inability to increase heart rate maximally or to lower vascular resistance further, as normally occurs with exercise (Graettinger et al., 1959; Mintz et al., 1991; Klein and Ojamaa, 1998). Occasionally patients with severe, long-standing hyperthyroidism have poor
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cardiac contractility, low cardiac output, symptoms and signs of heart failure, including a third heart sound and pulmonary congestion. This complex of findings most commonly occurs with persistent sinus tachycardia or atrial fibrillation and is the result of so-called rate-related heart failure (Magner et al., 1988; Cacciatori et al., 1996; Klein and Ojamaa, 1998).

In older patients with heart disease, the increased workload that results from hyperthyroidism may further impair cardiac function (Polikar et al., 1993). The presence of ischemic or hypertensive heart disease may compromise the ability of the myocardium to respond to the metabolic demands of hyperthyroidism (Graettinger et al., 1959; Klein, 1990). Prompt recognition and effective management of cardiac as well as other organ-system manifestations in patients over 50 years of age are important, because cardiovascular complications are the chief cause of death after treatment of hyperthyroidism (Franklyn et al., 1998). Alterations in cardiac hemodynamics have been reported in some, but not in all studies of patients with subclinical hyperthyroidism (Padayatty, 1998; Mercuro et al., 2000). The alterations include an increase in heart rate and left ventricular mass.

Sundaram et al., (1997) have also indicated that LDL oxidation is enhanced in hyperthyroidism compared to euthyroidism, which was unexpected. Further, the lag phases and propagation of LDL oxidation did not seem to be affected by drug therapy as long as the underlying thyroid condition was still present. However, Fernandez et al., (1985) were able to show that in experimental hyperthyroid rats, there was an increased generation of oxygen free radicals and increased activity of NADH-cytochrome P450 reductase and NADPH oxidases in rat liver microsomes. These effects were accompanied by increased levels of lipid peroxides, suggesting the disruption of polyunsaturated fatty acids by free radical oxidation. A similar mechanism could explain the increased susceptibility of LDL oxidation in hyperthyroid individuals, in whom the increased free radical generation overwhelms the antioxidant factors protecting the LDL. The significance of
enhanced LDL oxidation in hyperthyroid individuals is not clear. Hyperthyroidism is known to precipitate angina and myocardial infarction in patients with preexisting coronary heart disease, presumably secondary to the increased metabolic demands on the myocardium. However, the increased angina and precipitation of myocardial infarction could relate to the oxidative modification of LDL by free radicals, which, in turn, would cause changes in the endothelium-dependent relaxation factors. Interestingly, hyperthyroidism reduces the LDL cholesterol concentration without modifying HDL or triglyceride concentrations. It is possible that the effects of LDL oxidation may require a threshold level of LDL to induce atherosclerosis.

**Cellular Effects of Thyroid Hormones on Cardiovascular System**

Thyroid hormones may exert both genomic and nongenomic effects on cardiac myocytes. The genomic effects of thyroid hormones are mediated by the transcriptional activation or repression of specific target genes. These genes encode important structural and regulatory proteins, that encode both structural and functional proteins (Dillmann, 1990). This process begins with the entry of triiodothyronine (T₃), the biologically active thyroid hormone, into the cardiomyocyte through two T₃-binding nuclear receptors, TRα1 and TRβ1 that are present in the cardiac myocyte cell membrane (Everts et al., 1996). Once in the cardiomyocyte, T₃ enters the nucleus and interacts with specific transcriptional activators to modify the rate of transcription of specific target genes (Brent, 1994). Among various proteins whose expression is modulated at transcriptional level, the most-extensively characterized are myosin heavy chains (Morkin, 1993; Ojamaa et al., 1996b) and the sarcoplasmic reticulum protein involved in the regulation of intracellular calcium handling, namely, calcium-activated ATPase and its inhibitory cofactor, phospholamban (Dillmann, 1990; Kiss et al., 1994). Sarcoplasmic reticulum calcium-activated ATPase is responsible for the rate of calcium reuptake into the lumen of the sarcoplasmic reticulum during diastole that in turn is a major determinant of the velocity of myocardial relaxation after
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contraction (Dillmann, 1990; Kiss et al., 1994). However, the performance of sarcoplasmic reticulum calcium-activated ATPase is influenced by the level of expression of phospholamban: the higher the phospholamban expression, the lower the sarcoplasmic reticulum calcium-activated ATPase activity (Kiss et al., 1994). In this regard, it has been extensively demonstrated that thyroid hormones upregulate expression of the sarcoplasmic reticulum calcium-activated ATPase and down regulates expression of phospholamban, thereby enhancing myocardial relaxation (Dillmann, 1990; Kiss et al., 1994). Indeed, the improved calcium reuptake during diastole may favorably affect myocardial contractility. In fact, the greater reduction in cytoplasmatic concentration of calcium at end-diastole increases the magnitude of the systolic transient of calcium that, in turn, augments its availability for activation of tropo-myosin units. In phospholamban deficient mice, cardiac contractility was found to be increased, with no further increase after thyroid hormone treatment (Kiss et al., 1998). This finding strongly supports the key role of sarcoplasmic reticulum proteins and their effects on intracellular calcium handling in thyroid hormone-mediated changes in systo-diastolic cardiac function in patients with thyroid dysfunction. In this context, it is important to recognize that thyroid hormones also modify the expression of other ion channels, such as Na⁺/K⁺-activated ATPase, Na⁺/Ca⁺⁺ exchanger, and some voltage-gated K⁺ channels (Kv1.5, Kv4.2, Kv4.3), thereby coordinating the electrochemical and mechanical responses of myocardium (Gick et al., 1990; Ojamaa et al., 1999).

Evidences indicate that thyroid hormones promote the acute phosphorylation of phospholamban and that this action attenuates the inhibitory effect of phospholamban on sarcoplasmic reticulum calcium-activated ATPase (Ojamaa et al., 2002). Interestingly, the fact that this process is mediated at least in part by the activation of intracellular kinase pathway involved in signal transduction of the adrenergic stimulus (Ojamaa et al., 2002) may help to explain functional analogies between the cardiovascular effects of thyroid hormones and those promoted by the adrenergic system (Levey and Klein, 1990). Indeed, most of
the cardiovascular manifestations associated with hyperthyroidism and hypothyroidism mimic a condition of increased and reduced adrenergic activity. The sensitivity of cardiovascular system to adrenergic stimulation does not seem to be substantially altered in these conditions (Hoit et al., 1997; Ojamaa et al., 1999).

**Thyroid Hormones and LDL-R**

Molecular mapping has revealed functional thyroid response elements in the promoter region of LDL receptor. When the LDL receptor promoter was linked to a reporter gene and cotransfected with the β1 isoform of the thyroid hormone receptor into a hepatic cell line, specific stimulation by T₃ of this chimeric gene’s activity was observed (Bakker et al., 1998). Furthermore, deletion of the upstream thyroid response elements in LDL receptor promoter inhibited T₃-mediated reporter gene activity. Substantial evidence links the hypothyroid state with elevations of total and LDL cholesterol levels (Althaus et al., 1988; Caron et al., 1990). The elevated LDL cholesterol levels in hypothyroidism may occur as a result of increased cholesterol synthesis, and defects in the LDL receptor-mediated catabolism of LDL (Abrahm & Grundy, 1981). In contrast to hypothyroidism, euthyroid state and hyperthyroidism is usually associated with low total and LDL cholesterol levels (Kinlaw, 1995; Ganotakis et al., 2003). The cardiac abnormalities in this condition, i.e. angina and congestive heart failure, are thought to be secondary to the hyper metabolic state (Fernandez et al., 1985). Early studies in humans with hypothyroidism, using isotopically labeled LDL, demonstrated a prolonged half-life of LDL cholesterol because of decreased catabolism of LDL through LDL-R, an effect that was reversible with T₄ therapy (Walton et al., 1965). Studies in human fibroblasts verified that the T₃-induced increase in LDL degradation was mediated through an increase in LDL receptor number, without any change in the affinity of LDL for its receptor. Chait et al., (1979) have specifically suggested the effect of thyroid hormones on LDL receptor by a lack of T₃ effect on LDL concentration in cultured cells without LDL receptors. These findings were supported by an in vivo study in a hypothyroid woman whose
receptor-mediated LDL catabolism was reduced, compared with euthyroid controls, with significant improvement after T4 replacement therapy (Thompson et al., 1981). Further studies in rats with propylthiouracil-induced hypothyroidism showed a 50% reduction in LDL receptor mRNA levels (Staels et al., 1990, Salter et al., 1991).

In hypothyroidism the receptor mediated catabolism of LDL is markedly reduced, secondary to the down regulation of the cell surface LDL-R level (Thompson et al., 1981). Goldstein et al., (1983) have demonstrated that high fat diet, and deficiency of thyroid hormone raise plasma cholesterol levels by causing a partial suppression of LDL-R in the liver. In human plasma, thyroid hormones are transported primarily by T4-binding globulin, T4-binding prealbumin, and serum albumin. A small fraction (3%) of T4 is bound to plasma lipoproteins, with a relative distribution of 0.8% to very low-density lipoprotein, 6.7% to LDL, and 92% to HDL. T3 binds to the same proteins, but with a lower affinity (Benvenega et al., 1988; Benvenega and Robbins, 1990). The T4-LDL complex is recognized by the LDL receptor, and this interaction provides an additional mode of T4 entry into the cells (Benvenega and Robbin, 1990). Thus, the lipoprotein-bound T4 could be involved in protecting LDL from oxidation. Hanna et al., (1993) have suggested that in vitro, L-T4 functions as an antioxidant and inhibits Cu^{2+}-mediated LDL oxidation in a concentration-dependent fashion.

**Type-I 5’-Iodothyronine Deiodinase (5’-DI)**

Virtually all of the metabolic and developmental effects of thyroid hormones are mediated by T3, which is produced from T4 by 5’-deiodination (Geyten et al., 2005). Conversion of the prohormone thyroxine (T4) to the biologically active hormone, 3,5,3’-triiodothyronine (T3) is the first step in thyroid hormone action. The whole thyroxine circulating in the body is obtained from thyroidal synthesis, but over 80% of circulating T3 is derived from deiodination of T4 in peripheral tissues. Liver and Kidney are generally considered to provide
most of plasma T₃, but skeletal muscle may also contribute significantly to the plasma pool of T₃ (Leonard and Visser, 1986; Alvarez et al., 2005).

Practically all the tissues are capable of deiodinating T₄, and three general classes of deiodinase enzymes (Leonard and Visser, 1986; Solis et al., 2004) type-I, type-II and type-III catalyze this reaction. These enzymes are distinguished by their tissue distribution, physiological roles, Km for substrate and sensitivity to PTU (6-n-propyl-2-thiouracil). Type-I is the major enzyme in liver, kidney and skeletal muscle, it can carry out both 5’- and 5-deiodination of T₄, to produce T₃ and rT₃. The type-II enzyme is the major deiodinase in brain, pituitary and brown adipose tissue; this appears to carry out only 5’-deiodination. Type-II is especially important in providing the brain with T₃ and also for providing nuclear T₃ in the pituitary to control TSH synthesis and secretion. Most of the T₃ binding to nuclear receptors in brain and pituitary is derived from intracellular deiodination of T₄ rather than by uptake from plasma (Silva et al., 1978; Kaplan, 1984). The central nervous system contains a type-III deiodinase enzyme, which metabolizes T₄ to rT₃ (Hennemann, 1986). Type-I 5’-iodothyronine deiodinase (5’-DI) is the most important among all the three, as it provides most of the plasma T₃.

Behne et al., (1990) have concluded that type-I 5’-iodothyronine deiodinase is a selenoprotein. Se is situated at its active site as selenocysteine. The identity of 5’-DI as selenoprotein was also proved by Arthur et al., (1990) and later confirmed by cloning of the gene by Berry et al., (1991a) showing that mRNA for the protein contained a UGA codon for selenocysteine. Replacement of selenocysteine by cysteine or other amino acids lead to marked decrease in the enzyme activity (Low and Berry, 1996). Further confirmation that selenocysteine was present at its active site, came from studies showing that the wild type enzyme was quite sensitive to inhibition by gold thioglucoce, whereas cysteine containing enzyme required 100 fold higher concentration of gold for 50% inhibition. Gold is a much better inhibitor of selenocysteine than cysteine containing enzyme (Chaudiere and Tappel, 1984)
Type-I 5'-iodothyronine deiodinase is present predominantly in liver and kidney. It is an integral membrane protein and is located in the cytosolic side of endoplasmic reticulum in liver (Korhle, 1994) while in the kidney and LLC-PK1 cells, it is present in plasma membrane (Leonard and Rosenberg, 1978a; Leonard et al., 1991). More recently, a basolateral plasma membrane location was confirmed in glial cells constitutively expressing a green fluorescent protein-tagged 5'-DI (Leonard et al., 2000). Toyoda et al., (1994) have further suggested that 5'-DI protein is oriented with a 12-amino acid NH2-terminal extension in the endoplasmic reticulum (ER) lumen and a single transmembrane domain existing in the ER at about position 36. The hydrophobic nature of the NH2 terminus suggests that this portion of the molecule is an uncleaved signal recognition sequence and incorporates both signal and STOP-transfer functions. This orientation is in agreement with earlier studies showing that gentle trypsinization of kidney microsomes caused the loss of enzyme activity (Leonard and Rosenberg, 1978b; Macial et al., 1979; Leonard et al., 1991). Studies of the in vitro-translated Sec126 Cys mutant of rat 5'-DI showed that, although the NH2-terminal and transmembrane portions of the enzyme are not catalytically active, their sequence is critical because even minimal exchanges of amino acids in the transmembrane domain reduced the efficiency of its transient expression.

Much of the biochemistry of 5'-DI was established prior to the cloning of the enzyme using the alkylating substrate analogs, N-BrAcT4 and N-BrAcT3 (Mol et al., 1984b; Kehrle et al., 1990). Affinity labeling studies done with liver and kidney membrane preparations identified a 27kDa thyroid hormone-binding protein (p27) (Kehrle et al., 1990; Safran et al., 1990) that was subsequently cloned by functional expression (Berry et al., 1991a). Interestingly, molecular sieve chromatography of the detergent soluble rat kidney 5'-DI yielded a 50kDa functional enzyme (Leonard et al., 1981), and subsequent work showed that the affinity labeled p27 co-migrated with catalytic activity as a 54kDa complex (Leonard et al., 1991). These data suggested that the catalytically active 5'-DI
enzyme is a homodimer of 27kD subunits, which contain selenocysteine at its active site (Behne et al., 1990; Korhle et al., 1990). Only one subunit is active and can catalyze deiodination reaction.

Type-I 5'-iodothyronine deiodinase substrate specificity and catalysis is determined by four amino acid residues (Phe-65, Cys-124, SeC-126 and His-174) (Berry et al., 1991b; Berry et al., 1992; Toyoda et al., 1994; Croteau et al., 1998). The role of Phe-65 in iodothyronine specificity and of SeC126 in catalysis are well established (Berry et al., 1991b; Toyoda et al., 1994), whereas the role of Cys-124 and His-174 in the catalytic reaction are less understood. For example, replacement of Cys-124 with alanine led to parallel 2-fold decreases in the $K_m$ for substrate and in catalytic efficiency when assayed at a fixed cofactor concentration (Sun et al., 1997). Traditional chemical modification studies established that histidine was essential for catalysis (Mol et al., 1984a; Goswami and Rosenberg, 1990), and subsequent mutagenesis and transient expression studies identified His-174 as one of these essential residues (Berry, 1992).

The complete cDNA sequence has been determined for rat, human, mouse, dog, chicken and tilapia 5'-DI proteins (Berry et al., 1991a; Mandel et al., 1992; Toyoda et al., 1994; Maia et al., 1995; Sanders et al., 1997; Geyten et al., 1997). To characterize the mRNA for 5'-DI, Berry et al., (1990) determined its molecular weight. Poly (A)$^+$ RNA was size fractionated on low melting temperature agarose, fractions were injected into Xenopus oocyte, and 5'-DI activity was determined. The highest level of 5'-DI activity was encoded by the fraction containing mRNA between 1.9-2.4kb indicating that mRNA for 5'-DI is in this size range. Results by Mandel et al., (1992) demonstrated that the human 5'-DI gene and protein are highly homologous to those of rat. The coding region, nucleotide sequence of the two species are 82% identical and the putative amino acid sequences show 88% homology.

Studies with both endogenous and recombinant enzymes indicate that the deiodination reaction catalyzed by 5'-DI follows ping-pong kinetics with two
substrates, the first being the iodothyronine, the second being an endogenous intracellular thiol cofactor (Chopra, 1978; Leonard and Rosenberg, 1978b; Visser et al., 1978; Leonard and Rosenberg, 1980; Berry et al., 1991b). The first half-reaction deiodinates the iodothyronine leading to the formation of a putative selenoleyl iodide intermediate. This is then reduced by intracellular thiol cofactor regenerating the enzyme. 6-n-propyl-2-thio-uracil (PTU) inhibits 5'-DI catalyzed deiodination by competing with the putative thiol co-substrate to form an essentially irreversible enzyme-Se-S-PTU. Comparison of 5'-DI enzymes of different species has led to the recognition of other structurally important amino acids. For example, the phenylalanine at position 65 is critically important for 5'-deiodination of rT3 and 3,3'-diiodothyronine sulfate (T2S) but not for deiodination of substrates with two iodines on the inner ring (Toyoda et al., 1994).

**Apolipoprotein B and Its Implications on Cardiovascular Disorders**

Apolipoproteins are amphipathic in nature i.e. they have both hydrophobic and hydrophilic regions, and can therefore interact both with the lipids of the lipoprotein and with the aqueous environment (Segrest et al., 1994; Whitfield et al., 2004). Because of this nature apolipoproteins act as detergents, and have a major role in determining and stabilizing the size and structure of lipoprotein particles.

Apolipoprotein B (apoB) is the major structural apolipoprotein found mainly in the atherogenic lipoproteins, LDL and VLDL. The mature liver derived apoB-100 protein consists of 4536 amino acids plus a 27 amino acid leader sequence (Carlsson et al., 1986). The intestinal derived apoB-48 has a molecular mass of 210kDa and appears to correspond to the n-terminal 48% of apoB-100. Basically apoB contains the ligand-binding domain for the binding of LDL to LDL-R site, which enables the removal of LDL from the circulation. Much attention has been focused on understanding the molecular interaction between apoB-100 and the LDL receptor. The structural and functional domains of the LDL receptor have been defined in detail (Hobbs et al., 1992), but much less is
understood about the receptor-binding domain of apoB-100, because of its large size and insolubility in aqueous buffer. Furthermore, because apoB-100 binds to LDL receptor only after the conversion of large VLDL to smaller LDL (Brown and Goldstein, 1985), both its lipid composition and its conformation appear to be crucial to its function as an effective ligand for the LDL receptor. Although the exact receptor-binding site has remained elusive, several lines of evidence have suggested that residues 3,000-3,700 are critical for mediating apoB-100 binding to the LDL receptor (Milne et al., 1989).

The interaction between LDL and the LDL receptor plays a major role in determining plasma cholesterol levels in humans and other mammalian species (Brown and Goldstein, 1985) and apoB plays the central role in this ligand-receptor interaction. The relevance of this fact is best illustrated by the genetic disorder familial defective apoB-100 (FDB), in which high levels of LDL accumulate in the circulation because mutations in the ligand disrupt the binding of LDL to its receptor (Innerarity et al., 1990; Kaiser et al., 2002). Familial defective apoB-100 is associated with a single site mutation, the substitution of glutamine (Innerarity et al., 1987; Dedoussis et al., 2004), or, in a few cases, tryptophan (Gaffney et al., 1995) for the normally occurring arginine at residue 3,500 of apoB-100 is associated with a decrease in LDL receptor binding. The FDB mutation occurs at an estimated frequency of 1/500 in the normal population and is therefore one of the most common single-gene defects known to cause an inherited disorder (Innerarity et al., 1990).

ApoB is constitutively synthesized in the liver (Thomas et al., 1989). However, 40–60% of newly synthesized apoB is degraded rather than secreted (Borchardt and Davis, 1987). Consequently, the rate of apoB secretion is largely determined by the ability of newly synthesized apoB to escape degradation (Dixon and Ginsberg, 1993; Yao et al., 1997). In addition to LDL-receptor binding, apoB is known to interact with proteoglycans, a process also relevant in the pathogenesis of atherosclerosis (Williams and Tabas, 1995). Goldberg et al., (1998) showed that
apoB-48 was capable of binding to proteoglycans, explaining why apoB-48-containing lipoproteins may be equally atherogenic. Proteoglycans exist on the endothelial cell surface as well as inside the intima. Proteoglycans after binding to the endothelium may facilitate lipoprotein entry into the vascular intima and that in turn leads to plaque progression.

However, most of the recent studies have suggested that one molecule of apoB exists per lipoprotein particle, and thus the quantity of apoB in fasting plasma is a measure of the number of LDL and VLDL particles (Vega et al., 1990; Levinson and Wagner, 1992). Therefore, plasma apoB levels may be a better assay of the concentration of atherogenic lipoprotein particles than total or LDL cholesterol levels (Brunzell et al., 1984; Sniderman and Silberberg, 1990). Furthermore, a cross-sectional study in patients who had coronary artery bypass graft surgery determined that apoB concentration was a better discriminator than LDL cholesterol concentration in predicting recurrent atherosclerotic disease in bypass grafts 10 years after surgery (Campeau et al., 1984). Mutations in the apoB gene can cause low levels of apoB and LDL cholesterol and may be associated with protection from premature coronary artery disease (Linton et al., 1993). In another study, apoB levels were substantially higher in children of parents with premature coronary artery disease (Kostner et al., 1991). Wilcken et al., (1993) reported that apoB levels in children were predictive of premature coronary artery disease in their grandparents.

Salonen et al., (1985) have not observed any significant change in apoB levels in patients having high cholesterol levels, who died of coronary heart disease. However, these results may be difficult to extrapolate to other populations because of the exceptionally high cholesterol levels and coronary artery disease rates in these persons. In a prospective case-control study, Stampfer et al., (1991) found that plasma apoB level was highly correlated with the risk of coronary artery disease and was more predictive than total cholesterol levels. Coleman et al., (1992) in women found that although there was a positive correlation between

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apoB levels and coronary artery disease, the correlation with total cholesterol level was stronger. A large prospective study in 21,520 men found that apoB levels were considerably higher in 229 men who died of coronary artery disease than in the 1145 matched controls (Wald et al., 1994).

Disproportionately elevated plasma levels of apoB are characteristic of a syndrome called familial combined hyperlipidemia. This condition often is associated with other plasma lipid abnormalities, including mild to moderate hypertriglyceridemia and low HDL cholesterol levels (Goldstein et al., 1973; Rose et al., 1973). The plasma LDL cholesterol concentrations in these persons often are only modestly elevated. A related condition is hyperapobetalipoproteinemia (Sniderman et al., 1980; Whitfield et al., 2005), in which levels of plasma apoB increased but LDL cholesterol levels were normal. These syndromes, for which an elevated plasma apoB level is an important diagnostic criterion, are associated with a substantially increased risk for premature coronary artery disease (Goldstein et al., 1973; Sniderman et al., 1980). Quantitation of plasma apoB concentrations may be one method to identify these persons whose lipid parameters often are not especially abnormal. Patients with elevated fasting triglyceride levels or decreased HDL cholesterol levels may have familial combined hyperlipidemia. The plasma apoB concentration may be useful in differentiating these persons from those with other causes of dyslipoproteinemia, such as familial hypertriglyceridemia, which are not associated with increased risk for premature coronary artery disease (Brunzell et al., 1984). A recent study suggested that an elevated apoB level associated with elevated triglyceride levels might be more atherogenic than an increased apoB level alone (Kwiterovich et al., 1993).

Abnormalities in the metabolism of apoB containing lipoproteins are responsible for the generation of hyperlipidemia and the associated increased risk of developing coronary heart disease (Ouguerram et al., 2004; Whitfield et al., 2004). Studies by Teng et al., (1986) have revealed that subjects with the disorder hyperapobetalipoproteinemia had a reduced clearance rate and higher production
of VLDL and LDL particles. Davis and McNeal (1985) have found that dietary cholesterol did not affect the synthesis of apoB levels in rat hepatocytes. Tatsuda et al. (1987) and Esumi et al., (1990) have shown a positive correlation between serum apoB levels and atherosclerotic conditions. Abraham et al., (1993) have found a significantly increased production of apoB levels in cultured hepatocytes isolated from rats fed with atherogenic diet. The fact that the viability and attachment of cells isolated from both normal and atherogenic rat livers were comparable suggests that the difference in hepatic lipoprotein secretion observed here is not due to any inherent difference in collagenase dispersion of livers from control and experimental animals. Kosykh et al., (1988) have observed increased synthesis of apoB in cholesterol loaded rabbit hepatocytes. Teramoto et al., (1987) have demonstrated that cholesterol feeding in rats increased the production of VLDL several fold but the apoB level was not affected. Kumar et al., (1992) have demonstrated that incubation of hepatocytes isolated from normal rats with added cholesterol resulted in an increased synthesis and secretion of apoB levels. They further analyzed that suppression of cholesterol synthesis by inhibitors of HMG-CoA reductase had been found to decrease the synthesis and secretion of apoB (Cianflone et al., 1990).

3-Hydroxy-3-Methylglutaryl Co-enzyme A (HMG CoA) Reductase and Cholesterol Homeostasis

Genetic variability in susceptibility to the serum cholesterol raising action of dietary cholesterol has widely been suspected. The molecular mechanisms underlying these genetically determined differences are not established. Decreased expression of hepatic low-density lipoprotein (LDL) receptor or mutations in apoB or apoE are well-known to cause increases in serum cholesterol levels (Hoffman et al., 1988; Soria et al., 1989). In addition to this, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the enzyme that catalyzes the rate-limiting step in cholesterol biosynthesis, also plays a significant role in cholesterol homeostasis (Ness and Chambers, 2000). This enzyme is the target for statin class
of cholesterol-lowering drugs that are currently being used so effectively (Bedi and Flak. 2002; Muhlestein, 2004). Eukaryotic HMG-CoA reductase is an eight-transmembrane span protein with a cytosolic catalytic domain anchored to the endoplasmic reticulum (Roitelman et al., 1992).

Cellular cholesterol homeostasis is maintained primarily through regulation of the LDL receptor and HMG-CoA reductase, which affect exogenous uptake and endogenous synthesis, respectively, of cholesterol (Brown and Goldstein, 1986). Cholesterol uptake by the LDL receptor is generally considered to be regulated via transcriptional control mechanisms (Goldstein and Brown, 1977; Brown and Goldstein, 1986; Cuthbert and Lipsky, 1990) whereas HMG-CoA reductase activity is regulated at many levels, from transcriptional control to degradation of the enzyme (Harwood et al., 1987; Goldstein and Brown, 1990; Ness et al., 1991). A large number of studies have elucidated the role of these regulatory mechanisms in vitro, in experimental animals, and in humans treated with experimental diets or drugs (Brown and Goldstein, 1986; Goldstein and Brown, 1990). However, very little is known about the transcriptional regulation of cholesterol metabolism in peripheral cells from healthy subjects in vivo and its inherent physiological inter individual variations, i.e. without induction of specific alterations by diet or drugs. Such evaluations are important to estimate the significance of the various regulatory mechanisms under physiological conditions in vivo, where the high concentration of LDL cholesterol in human plasma induces a very strong down regulation of LDL receptor and HMG-CoA reductase expression (Ho et al., 1976; Ho et al., 1977; Trail et al., 1987; Cuthbert and Lipsky, 1992). One reason for the lack of information regarding the physiological regulation of cholesterol metabolism may lie in the need for more highly sensitive and accurate methods by which minimal amounts of specific mRNAs can be reliably quantified.

HMG-CoA reductase was shown to be coordinately regulated with the LDL receptor at the transcriptional level (Goldstein and Brown, 1990; Rudling, 1992; Spady and Cuthbert, 1992). Powell and Kroon (1994) have shown a comparable
coordinate transcriptional regulation of the LDL receptor and HMG-CoA reductase in peripheral blood mononuclear cells and liver samples from middle-aged donors. However, the factors involved in transcriptional regulation of HMG-CoA reductase in vivo are only partly identical to those for LDL receptor regulation, because in contrast to HMG-CoA reductase, LDL receptor mRNA is regulated by plasma concentrations of LDL and HDL (Powell and Kroon, 1994). Factors other than sterols have also been shown to be involved differently in transcriptional regulation of the LDL receptor and HMG-CoA reductase, e.g. mitogenic signals (Cuthbert and Lipsky, 1992) and intracellular calcium respectively (Roth et al., 1993).

A striking correlation between basal expression of hepatic HMG-CoA reductase and resistance to dietary cholesterol was demonstrated by Ness and Gertz (2004) in the 9 inbred rat strains and the Golden Syrian hamsters. Animals with the highest hepatic HMG-CoA reductase activity were completely resistant to dietary cholesterol. The Golden Syrian hamsters expressed the lowest level of hepatic HMG-CoA reductase activity and exhibited by far the greatest increase in serum cholesterol levels when given a cholesterol challenge. These results are in agreement with the observations made in the Scandinavian Simvastatin Survival Study ("4S study") of patients on simvastatin (Miettinen et al., 2000).

The data in the study by Ness and Gertz (2004) provided strong support for the concept that hepatic HMG-CoA reductase functions as a cholesterol buffer (Ness and Chambers, 2000). When animals face a cholesterol challenge, they respond by markedly downregulating the expression of hepatic HMG-CoA reductase protein. Thus, animals that express high basal levels of hepatic HMG-CoA reductase are resistant to dietary cholesterol, whereas those expressing low levels of reductase (hamsters) are very susceptible to the serum cholesterol raising action of dietary cholesterol.
Management of Hypercholesterolemia

Management of hypercholesterolemia and in turn cardiovascular disorders is also a major thrust area of research. Atherosclerosis represents a state of oxidative stress characterized by lipid and protein oxidation (Stocker and Keaney, 2004). Also, free radicals are known to play an important role in the initiation of plaque formation and involvement of antioxidants as the preventive agents in cardiovascular diseases has been implicated. One such well-known potent antioxidant is the trace element, selenium (Se). Wojcicki et al., (1991) have reported the protective action of Se against atherosclerosis. Studies have demonstrated the association of Se deficiency with coronary heart disease (Salonen et al., 1985; Salonen et al., 1988; Huang et al., 2002). Also the protective role of Se supplementation during MI (Myocardial Infarction) has been reported (Poltronieri et al., 1992).

Selenium: An Essential Nutrient

The essential role of selenium in nutrition has been well established. Selenium is an important trace element for humans and many other forms of life. Although initially selenium was considered to be a potent toxicant with pathological changes such as necrosis, hemorrhage, oedema etc in livestock exposed to high concentration (Franke, 1934; Rosenfield and Beath, 1964. Shortridge et al., 1971). However, later it was perceived that selenium was an essential requirement of the body at dietary levels of less than one ppm (Schwarz and Foltz, 1957). Deficiency of this element induces various pathological conditions in addition to coronary heart disease such as cancer, and liver necrosis (Burk et al., 1980; Salonen et al., 1991; Suadicani et al., 1992; Clark et al., 1996; Allan et al., 1999). Selenium deficiency is accompanied by a loss of immunocompetence (Field et al., 2002) and both cell-mediated immunity and B-cell function were seen impaired (McKenzie et al., 1998). Supplementation of selenium has marked immunostimulant effects, including an enhancement of activated T-cell proliferation (Schumacher et al., 1994). Selenium also has a
protective role against some toxic metals, Miyazaki et al., (2005) demonstrated that selenium deficiency leads to accumulation of arsenic in pregnant mice. Selenium is an essential component of several enzymes such as glutathione peroxidase (Takahashi et al., 1987), type-I 5′-iodothyronine deiodinase (Behne et al., 1990), thioredoxin reductase (Yarimizu et al., 2000), and selenoprotein P (Yang et al., 1987), which contain selenium as selenocysteine.

It is also well known that selenium is necessary for cell culture when using a serum-free medium. Selenites are added to the serum-free media for immune and neuronal cells. Saito et al., (2003) have observed a decrease in cell viability in selenium deficient medium in Jurkat cells. This cell death was completely blocked by selenium supplementation in a dose dependent manner. Many reports have shown significant correlations between selenium deficiency and the incidence of cancer (Knekt et al., 1998; Yoshizawa et al., 1998). It has also been reported that selenium supplementation prevents the generation of cancer (Clark et al., 1996; Lillico, 2003). Selenium deficiency caused a significant increase in ROS and peroxidation inside the cells. It is therefore considered that selenium-deficient conditions cause oxidative DNA damage that eventually leads to cancer formation.

Other studies have suggested that Se deficiency is related to adverse outcomes of pregnancy. Barrington et al., (1996) have shown that maternal blood Se levels were low in women who experience a first trimester miscarriage, when compared with women at the same stage of pregnancy who carry to term. Maternal Se levels were also found to be decreased in women with preterm deliveries and glutathione peroxidase level was diminished in the premature infants (Dobrzynski et al., 1998). Although it has been shown that severe Se deficiency can affect fertility in both male and female rodents (Bedwal and Bahuguna, 1994) but rats can be carried through three generations on a diet low in Se (Meinhold, 1993). In some reports, however, significant abnormalities involving sparse hair, delayed growth, retarded motor skills, and ocular abnormalities have been observed in the Se-deficient offsprings (Mckoy et al., 1969; Watanbe and Satoh, 1994).
How Selenium Functions in the Body

Selenium functions as a cofactor of 2 functionally distinct enzymes: glutathione peroxidase (Rotruck et al., 1973; Madipatti and Marnett, 1987) and type-I 5'-iodothyronine deiodinase (Mandel et al., 1992). Selenium-dependent glutathione peroxidase and other antioxidants are involved in the elimination of free radicals and reactive oxygen species (ROS). Highly reduced nutritional intake of selenium may impair enzyme activity and thus have deleterious effects not only on cells and organs, but also on the whole organism (Knight, 1995; Ip, 1998). Type-I 5'-iodothyronine deiodinase (5'-DI) as mentioned above is responsible for the conversion of the prohormone thyroxine (T4 or 3',5',3,5-tetraiodothyronine) into the biologically active triiodothyronine (T3 or 3',3,5-triiodothyronine). Thyroid hormones are important for normal growth and development of the maturing organism (Smith, 1985).

Absorption of selenium in the mammalian system to a great extent depends on the chemical form and the mode of administration of the element. Selenomethionine is better absorbed than sodium selenite when administered orally (Swanson et al., 1991). Justification to this fact probably is that the bioavailability of selenomethionine would be much more than selenite. Under the normal feeding conditions, absorption is not a limiting factor to bioavailability (Mutanen, 1986). Selenium is available to the metabolic pathway only after selenomethionine is broken down, which is again affected by the selenium status of the organism (Burk et al., 1972). Selenomethionine was found to be actively absorbed by the same mechanism as methionine (Combs and Combs, 1984) whereas selenite is passively but rapidly absorbed. Immediately after administration, selenium is taken up by erythrocytes. Jenkins and Hidiroglou (1972) observed that selenite was metabolized in the erythrocytes by a glutathione dependent system to form hydrogen selenide or similarly reduced selenide that was then readily shifted to the plasma proteins. From plasma proteins, selenium enters in tissues including bone, hair and blood cells. Hierarchy of selenium...
retention was observed in different tissues, when depleted rats were supplemented with the trace element. Preferential accumulation of selenium in the vital organs like thyroid, brain, gonads, pituitary and adrenal tissue over liver, erythrocyte, heart and muscle was observed (Behne et al., 1988).

**Selenium Status and Cardiovascular Disorders**

A link between selenium status and cardiovascular disorders was originally proposed based upon the ecological studies in which the selenium content of crops and drinking water was related to its concentration in blood, and to the regional cardiovascular disease mortality rates (Schamberger et al., 1979). Salonen et al. (1991) have reported that the serum selenium concentrations may be related to the progression of carotid artery atherosclerosis. They have found that subjects from Eastern Finland with no previous evidence of coronary disease and very low serum selenium concentrations had an increased risk of myocardial infarction and death from ischemic heart diseases. A Danish cohort study reported a significant but weak association between serum selenium below 79 µg/L and the risk of coronary heart disease among subjects with no pre-existing history of coronary heart disease (Suadicani et al., 1992). However, Miettinen et al., (1983) were unable to show an association between ischemic heart disease and the serum selenium concentrations in a group from Helsinki. Virtamo et al., (1985) were unable to demonstrate a significant relationship between serum selenium and the risk of coronary heart diseases in a study based in rural areas of eastern and southwestern Finland.

Various studies on rat model indicated that selenium deficiency resulted in a significant increase in plasma cholesterol levels (Stone et al., 1986; Stone, 1988; Huang et al., 2002; Lee et al., 2003). Mazur et al., (1996) have shown that increase in plasma cholesterol level during selenium deficiency is due to marked increase in apolipoprotein E and HDL concentration. Nassir et al., (1997) have demonstrated that selenium deficiency lead to increased HMG-CoA reductase activity (rate controlling enzyme in the cholesterol biosynthesis) which inturn
resulted in increased endogenous cholesterol synthesis. Selenium deficiency endemics have been described in several parts of the world. Vijaya et al., (2000) have demonstrated that selenium deficiency is associated with cardiomyopathy (Keshan disease). Cardiomyopathy is characterized by multifocal myocardial necrosis resulting in congestive heart failure. Selenium supplementation proved beneficial in the above conditions (Skoloff, 1985). Oster and Prillwitz (1990) have shown that selenium deficiency is associated with cardiovascular disorders. Epidemiological studies by Jossa et al., (1991) have also favored the above said statements. Various investigators (Kok et al., 1989; Beaglehole et al., 1990) have shown that Se deficiency lead to cardiovascular risk and ischaemic heart disease.

Studies indicated the protective effect of selenium supplementation during myocardial ischemia (Poltronieri et al., 1992). Wojcicki et al., (1991) have reported decrease in total cholesterol and triglycerides levels along with an increase in HDL cholesterol fraction on selenium supplementation during hypercholesterolemia. Further they have suggested that selenium is more effective then vitamin E in protection against formation of atherosclerotic lesions in rabbits. They have reported that areas of atherosclerotic lesions in the total aorta were reduced 49% by selenium and 63% by vitamin E combined with selenium and were slightly (25%) but not significantly reduced by vitamin E alone. Kang et al., (2000) have demonstrated the protective role of selenium. they have shown that selenium supplementation upto 1ppm normalized the cholesterol and triglycerides level in SD male rats. Further they have suggested through their scanning electron microscopic studies that Se supplementation has protective effect on high fat induced changes on the surface architecture of aorta.

Schoene et al., (1984) and Huang et al., (2002) have shown an increased platelet aggregation, increased production of thromboxane-A2 (TXA2) in collagen stimulated platelets and decreased prostacyclin (PGI2) production in aorta of rats after selenium depletion. PGI2 is a powerful vasodilator and inhibitor of platelet activating factor (PAF) synthesis (Zimmerman et al., 1985). PAF is a potent
activator of neutrophils, monocytes/macrophages and platelets and thus of inflammation (Benvensite et al., 1975). Enhanced PAF production in the presence of decreased PGI₂ synthesis by endothelium of rats with decreased selenium levels may alter interaction of platelets, monocytes/macrophages and PMN with endothelial cells at vascular surface. These altered cellular interaction in turn may contribute both to the progression of atherosclerosis and to increased vascular occlusion in coronary disease affected vessels, resulting in increased atherosclerotic lesions (Salonen et al., 1982; Moore et al., 1984) associated with low selenium levels.

**Selenium Dependent Glutathione Peroxidase and Cardiovascular Disorders**

Many aspects of the pathogenesis of cardiovascular disorders have been unraveled in recent years and an important potential role of oxidative mechanisms has been elucidated (Ross, 1999; Glass and Witztum, 2001). Oxidative stress may be defined as an imbalance between the production and degradation of reactive oxygen species such as superoxide anion, hydrogen peroxide, lipid peroxides, and peroxynitrite. Enzymatic inactivation of reactive oxygen species is achieved mainly by glutathione peroxidase (GSH-Px), superoxide dismutase, and catalase (Forsberg et al., 2001). In mammalian cells GSH-Px constitute the principal antioxidant defense system (Raes et al., 1987; Ursini et al., 1995). Glutathione peroxidase, the ubiquitous intracellular form and key antioxidant enzyme within most cells, including the endothelium, uses glutathione to reduce hydrogen peroxide to water and lipid peroxides to their respective alcohols (Flohe, 1988), and it also acts as a peroxynitrite reductase (Sies, 1999). Glutathione peroxidase deficiency results in abnormal vascular and cardiac structure and function ( Forgione et al., 2002a).

Glutathione peroxidase deficiency has been shown to increase cell-mediated oxidation of low-density lipoprotein in mice (Guo et al., 2001). Furthermore, mice that are heterozygous for GSH-Px deficiency have endothelial dysfunction combined with structural vascular abnormalities, such as increased periadventitial
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inflammation, neointimal formation, and collagen deposition surrounding the coronary arteries (Forgione et al., 2002b). Glutathione peroxidase deficiency apparently decreases bioavailable nitric oxide (Forgione et al., 2002b), an effect that can be aggravated by hyperhomocysteinemia (Dayal et al., 2002). In addition, glutathione peroxidase activity is decreased or absent in carotid atherosclerotic plaques, and the lack of glutathione peroxidase activity in atherosclerotic lesions appears to be associated with the development of more severe lesions (Lapenna et al., 1998). Since catalase activity is absent from human vascular cells (Shingu et al., 1985) and superoxide dismutase is poorly effective against cellular oxidative damage (Raes et al., 1987), the most important antioxidative shield is reduced in atherosclerotic plaque.

Smoking consistently reduces glutathione peroxidase activity, whereas the effect of commonly used drugs appears to be negligible (Anderson et al., 1997). Cigarette smoking is strongly associated with dysfunctional vasomotor responses, diminished nitric oxide levels, and time-dependent decreases in the content of endothelial nitric oxide synthase messenger RNA (Su et al., 1998). In accordance with previous studies (Mullholand et al., 1999), glutathione peroxidase activity decreased in smokers and former smokers. However, the association between low levels of glutathione peroxidase activity and high cardiovascular risk was also observed in smokers. Therefore, measurement of glutathione peroxidase should identify smokers who are at highest risk for cardiovascular events.

Blakenberg et al. (2001) have shown that low erythrocyte glutathione peroxidase activity identifies patients with coronary artery disease who are at the highest risk for cardiovascular events. Further observations suggested that measurement of glutathione peroxidase activity provided additional information that might be useful in identifying patients who would benefit from preventive antioxidative treatment (Blankenberg, 2002; Blankenberg et al., 2003).
**Selenium Status: 5'-DI Activity and Thyroid Hormones**

Selenium plays an important role in the control of thyroid hormone metabolism through its dependent enzyme 5'-DI. Initial evidence from animal experiments, clinical and *in vitro* cell culture studies suggested a clear Se dependent expression of 5'-DI varying with Se availability (Kohrle, 1999). Selenium being integral part of this enzyme (Behne *et al.*, 1990, Berry *et al.*, 1991a), activity rapidly decreases in Se deficiency (Beckett *et al.*, 1989). Beech *et al.*, (1995) have demonstrated that rats fed on Se deficient diet had decreased hepatic 5'-DI activity when compared to Se supplemented animals. Beckett *et al.*, (1987) and Arthur *et al.*, (1990) have demonstrated that a single injection of selenium (200μg of Se/kg body weight, as Na2SeO3) in selenium deficient rats restored the hepatic 5'-DI activity within 5-8 days.

Studies in rats fed a Se-deficient diet have documented that levels of deiodinase activity were greatly decreased in liver and kidney but were maintained in brain, thyroid and placenta (Beckett *et al.*. 1989; Chanoine *et al.*. 1992; Chanoine *et al.*, 1993; Meinhold *et al.*, 1993). It has been suggested that the ability of a tissue to maintain levels of deiodinase activity is a function of the extent to which it can maintain its local Se concentration (Buckman *et al.*, 1993; Vadhanavikit and Ganther, 1993). Bates *et al.*, (2000) demonstrated that in liver, during Se deficiency, 5'-DI activity was greatly diminished, whereas in cerebrum, thyroid, and pituitary, the deiodinase levels were well maintained. They further demonstrated that the relationship between the decrease in Se content and tissue deiodinase activity seems to be independent of age. There was no evidence that the effects of Se-deficiency were greater in the fetus and neonate than in the adult. Olivieri *et al.*, (1995) have observed low T3 and high T4 levels in Se deficiency in elderly people with the age. These results supported the hypothesis that an impaired Se status may explain the hormonal changes observed in elderly people, conceivably via impaired activity of the Se containing enzyme, 5'-DI.
Aaseth et al., (1990) have demonstrated that the human thyroid contains approximately twofold higher concentrations of selenium than liver and in selenium deficiency in rats the thyroid retains selenium more effectively than other organs (Behne et al., 1988) indicating that the element may have important functions in the gland. Arthur et al., (1990) have shown that selenium deficiency caused significant decrease in total iodine, T₃ and T₄ content of thyroid without any change in thyroid weight, and thyroid glutathione peroxidase activity decreased by 50%. They further demonstrated that there are three mechanisms whereby selenium deficiency could affect the structure and function of thyroid gland as well as metabolism of thyroid hormones. First, by decreasing glutathione peroxidase activity in the thyroid and thus increasing the concentrations of hydrogen peroxide. GSH-Px is thought to be the main antioxidant system for neutralizing cytotoxic H₂O₂ and its oxidative by-products (Combs et al., 1975). Hydrogen peroxide is produced by thyroid as a cofactor in thyroid hormone synthesis (Dumont, 1971), so selenium being a part of Se-dependent glutathione peroxidase may also play an indirect role in the control of thyroid hormone synthesis. Second, selenium deficiency may adversely affect the 5'-DI activity in thyroid gland. Third, the changes in the thyroid hormones caused by selenium deficiency could affect the gland through alterations in the circulating concentrations of TSH (Arthur et al., 1991).

Pizzuli and Ranjbar (2000) have demonstrated that low selenium levels in children lead to hypothyroidism. They observed that there were no pathological findings in thyroid gland sonography, no biochemical parameters for infection, negative immunological markers and normal iodine excretion in 24hrs urine (in accordance with WHO criteria). This way they were able to exclude all commonly known causes of hypothyroidism, but they finally observed low selenium levels in all the children. It was concluded that hypothyroidism in their patients was due selenium deficiency. After 4 weeks of oral therapy with 10μg/kg/body-weight of
sodium selenite, both T<sub>3</sub>/T<sub>4</sub> and selenium levels had returned to normal. They had seen marked clinical improvement in their patients.

Arthur et al., (1988) have demonstrated that an increased plasma T<sub>4</sub> and decreased plasma T<sub>3</sub> occurred in selenium deficient cattle. The effects of Se deficiency are complex due to the reason that Se retention during dietary deficiency differs among different tissues high in brain, pituitary, thyroid, adrenals, and gonads. In contrast, dietary Se deficiency rapidly reduces the Se content of plasma, liver, skeletal muscle, and heart (Meinhold et al., 1992; Bermano et al., 1995; Bates et al., 2000). Thus, the effect of Se deficiency on the synthesis of intracellular selenoproteins, such as the selenodeiodinases and GSH-PX, will depend on the tissue being examined.

In kidney and liver, thyroidectomy and the resultant hypothyroidism caused the loss of 5′-DI activity in both selenium supplemented and selenium deficient rats (Leonard et al., 1991). Selenium deficiency further decreased 5′-DI in both euthyroid and hypothyroid rats suggesting that the effects of hypothyroidism and selenium deficiency were additive. 5′-DI activity decreased in hypothyroid state and elevated in hyperthyroidism (Larsen et al., 1981). Berry et al., (1990) observed that type-I deiodinase mRNA levels correlated with thyroid hormone (T<sub>3</sub>/T<sub>4</sub>) status. Contempre et al., (1991) have shown that in the areas like Africa where myxedematous critinism occurs due to low selenium and iodine intake, selenium supplementation without iodine prophylaxis resulted in a marked drop in serum T<sub>4</sub> concentrations through an increase in 5′-DI activity. Vanderpas et al., (1990) confirmed that the lower blood selenium concentration and glutathione peroxidase activity was associated with cretinism. Selenium supplementation to these subjects increased their selenium status, but the influence of treatment on thyroid hormone status was not reported. Selenium deficiency could influence the outcome of iodine deficiency in areas of endemic goiter through its role in thyroid hormone metabolism or through peroxide metabolism in the thyroid.
All the above said findings suggested that pathogenesis of hypercholesterolemia and inturn atherosclerosis is multifactorial. Different investigators have related cardiovascular disorders with thyroid enzyme (T₃/T₁) status, also various studies have related selenium deficiency with cardiovascular disorders. Type-I 5'-iodothyronine deiodinase (a selenoprotein) is important for circulating T₃ levels in the body. T₃ is involved in the regulation of LDL-R expression. So considering these facts we tried to explore in depth the behavior of 5’-DI and LDL-R at translational as well as transcriptional level during experimental hypercholesterolemia and under different selenium status in SD male rats.