**DISCUSSION**

Intracellular and extracellular cholesterol levels are tightly maintained within a narrow range by an intricate transcriptional control mechanism. Extracellular cholesterol transport is largely mediated by plasma lipoproteins. High cholesterol diet feeding leads to cholesterol deposition in the arterial wall (Castro et al., 2005). Defects in the cholesterol metabolism are the major cause of cardiovascular disorders (Pasha, 2005). Brown and Goldstein (1984) have demonstrated that much of atherogenicity in general population is caused by a dangerously high level of low-density lipoproteins (LDL) in blood resulting from failure to produce enough LDL-R. Enormity of hypercholesterolemia is associated with the variation in the LDL-R gene (Dedoussis et al., 2004; Nomura et al., 2005). Liu et al., (1997) demonstrated that on feeding high fat diet, the liver LDL-R mRNA expression decreased in mice. The LDL receptor mediates the removal of LDL and remnant lipoproteins from circulation by binding to apolipoprotein B. Loss of LDL receptor function leads to decreased LDL catabolism and elevated LDL levels.

Involvement of thyroid hormones ($T_3/T_4$) has also been reported in context of cardiovascular disorders (Arem and Patch, 1990; Pingitore et al., 2005), as hypothyroidism is said to be associated with the disease process (Jung et al., 2003). Hypercholesterolemia has been shown to induce hypothyroidism (Glueck et al., 1991). Vierhapper et al., (2000) have shown that the patients with subclinical hypothyroidism have elevated cholesterol levels. Canaris et al., (2000) and Pirich et al., (2000) in their studies of patients with subclinical hypothyroidism have shown that they were very much prone to induce hypercholesterolemia.

In hypothyroidism, the receptor-mediated catabolism of LDL is markedly reduced (Thompson et al., 1981). Goldstein et al., (1983) have demonstrated that high fat diet and deficiency of thyroid hormones raise plasma cholesterol levels by causing suppression of LDL-R in the liver. These studies support a plausible role
Discussion of hypothyroidism in increasing the risk of atherosclerotic cardiovascular diseases via increase in levels of highly atherogenic LDL cholesterol particles.

Basically $T_3$ is the biologically active form of thyroid hormones. All of the metabolic and developmental effects of thyroid hormones are mediated by $T_3$ that is produced from $T_4$ by 5'-deiodination. This reaction is catalyzed by type-I 5'-iodothyronine deiodinase (5'-DI) enzyme, mainly in the liver (Alvarez et al., 2005). Selenium being the integral part of this enzyme, the activity as well as expression changes along with the selenium status (Beckett et al., 1989). Further, owing to this Se dependent behavior of 5'-DI, it becomes evident that selenium is playing a direct role to maintain $T_3$ levels in the body. Thus selenium being an inherent component of 5'-DI is directly regulating the $T_3$ levels and in-turn it must be having direct or indirect role in regulating the LDL-R levels required for normal clearance of LDL from blood.

So, the present study was undertaken to explore the behavior of 5'-DI and LDL-R at translational as well as transcriptional level along with the associated parameters during experimental hypercholesterolemia under different selenium status.

Selenium Levels

In the present study, to achieve different selenium (Se) status, selenium deficient (0.02ppm), adequate (0.2ppm) and excess (1ppm) diet was fed to the rats for 1, 2 and 3 months. Selenium levels in liver and serum were estimated in all the groups at all the three treatment intervals. Frost and Lish (1975) have suggested that selenium level in liver is considered to be the selenium status of the animals. Significantly decreased levels of selenium were observed in liver and serum in selenium deficient groups (Ia & Ib) in comparison to adequate selenium fed groups (IIa & IIb). On high cholesterol diet (HCD) feeding in all the three groups, selenium levels decreased significantly (Table 2,3; Fig. 2,3). These results are in agreement to the earlier findings that selenium deficiency is associated with hypercholesterolemia and in turn cardiovascular disorders (Oster and Prellwitz.
Discussion

1990; Lee et al., 2003). In support of the present results, Subramanayam et al., (1998) have reported a decrease in serum selenium levels on high cholesterol diet feeding. Low selenium levels are associated with increased platelet aggregation and thromboxane A2 production along with decreased prostacyclin production (Huang et al., 2002). All of these may be linked with cardiovascular diseases (Salonen et al., 1988).

However, not much information about the effects of high dietary selenium status (more than adequate level) is available in literature. It is observed here that an increase in the selenium supply in the form of selenite above the adequate level lead to the elevated serum and tissue selenium concentrations (Whanger and Butler, 1988) and the retention is higher in the liver than serum (Table 2,3: Fig. 2,3). These increased selenium levels were further illustrated by increased glutathione peroxidase (GSH-Px) activity on selenium supplementation, since GSH-Px levels have been reported to increase along with the dietary selenium supplementation, till a saturation level is achieved (Whanger and Butler, 1988). Most animals have selenium requirement between 0.1 and 0.3mg Se/kg of diet. The selenium excess dose (1 ppm) chosen for the present study is in excess to the adequate level of selenium (0.2ppm) and is considered to be in the supra nutritional range (1-3mg Se/kg diet) but is well below the toxic level (above 3ppm).

**Glutathione Peroxidase**

Recently, prospective role of oxidative mechanisms has been elucidated in the pathogenesis of cardiovascular disorders (Ross, 1999; Glass and Witztum, 2001). Oxidative stress may be defined as an imbalance between the production and degradation of reactive oxygen species.

Selenium nutritionally acts through its dependent enzymes like glutathione peroxidase. Since the discovery that GSH-Px is a selenoprotein (Rotruck et al., 1973), there have been numerous reports using the activity of this enzyme as an indicator of nutritional status for the selenium. In the present results in selenium
deficient groups (Ia and Ib), hepatic GSH-Px activity significantly decreased in comparison to adequate diet fed animals (IIa & IIb). Also in selenium deficient control group along with the increase in the time interval of diet feeding i.e. after 2 and 3 months, the GSH-Px activity decreased in comparison to 1-month treatment interval (Table 4; Fig. 4). So these observations confirm the selenium deficiency (Arthur et al., 1993), which is associated with decreased GSH-Px levels. Glutathione peroxidase deficiency results in abnormal vascular and cardiac function (Forgione et al., 2002a). In fact, reduced expression of GSH-Px has been shown to increase cell-mediated oxidation of LDL (Guo et al., 2001). Furthermore, GSH-Px deficiency leads to endothelial dysfunction combined with structural vascular abnormalities, such as increased periadventitial inflammation, neointimal formation, and collagen deposition surrounding the coronary arteries (Forgione et al., 2002b). In addition GSH-Px activity is decreased or absent in carotid atherosclerotic plaques, and the lack of GSH-Px activity in atherosclerotic lesions appears to be associated with the development of more severe lesions (Lapenna et al., 1998).

It can be interpreted from our results that in all the three selenium status groups on high cholesterol feeding, inspite of decrease in selenium levels the GSH-Px levels increased at all the treatment intervals. Also, in group Ib and IIb (HCD fed. Se deficient and adequate groups), after 2 and 3 months, level of GSH-Px increased significantly in comparison to 1-month data (Table 4, Fig. 4). This increase in selenium dependent GSH-Px on high cholesterol diet feeding is attributed to the increased lipoperoxidative stress associated with HCD feeding. This is in support with the literature where elevation of GSH-Px activity is reported to be associated with small increase in oxidative stress (Oei et al., 1982; Kang et al., 2000). Also, in mammalian cells, glutathione peroxidase constitutes the principal antioxidant defense system (Raes et al., 1987; Ursini et al., 1995). This increase in the tissue Se-dependent GSH-Px on high cholesterol diet feeding
explains the decrease in selenium levels as observed in the present study, even though diet contains adequate level of selenium in high cholesterol fed group (IIb).

On 1ppm selenium supplementation significantly increased levels of GSH-Px were observed in the present study. In selenium-supplemented groups as the selenium deposition increased i.e. after 2 and 3 months, the GSH-Px levels increased in comparison to 1-month data (Table 4; Fig. 4). Selenium being the integral part of GSH-Px and the activity of this enzyme, to certain levels, is directly related to selenium availability (Scholz et al., 1994). Thus increased selenium levels in Se supplemented groups directly resulted in increase in the GSH-Px activity. Ip and Hayes (1989) reported that on feeding excess selenium (3mg /kg) as selenite in the diet over a period of 4 weeks, liver GSH-Px activity was found to be slightly elevated. Glutathione peroxidase was recently shown to inhibit 5-lipoxygenase in monocyctic cells (Straif et al., 2000). The 5-lipoxygenase is induced in monocytes and macrophages within progressing atherosclerotic lesions (Spanbroek et al., 2003) and strongly contributes to atherosclerotic susceptibility (Mehrabian et al., 2002). The interference of glutathione peroxidase with 5-lipoxygenase might constitute a protective function of the enzyme, in addition to its antioxidant activity. A further pathway by which glutathione peroxidase may have an effect on atherogenesis is through its direct effect on the immune system, as atherogenesis is considered to be a state of hyper inflammation. Glutathione peroxidase appears to modulate the oxidative products of the respiratory burst of phagocytic cells. Selenium deficiency leads to decreased GSH-Px levels, which is associated with increased hydrogen peroxide production (Li and May, 2002) and superoxide production in macrophages (Spallholz and Boylan, 1989), while dietary selenium supplementation reduces this process (Allen, 1986). The reduction in hydrogen peroxide and superoxide would reduce oxidation of LDL, and thus have a favorable effect on atherogenesis.
Cholesterol and Triglyceride Levels

Excess of lipids in the serum derived from endogenous synthesis/dietary sources initiate atherosclerosis by accumulation in the cells of arterial wall and provoking atheroma growth (Palmer et al., 2004). In the present study in all the selenium status groups on high cholesterol diet feeding, the cholesterol and triglycerides levels increased significantly after 1, 2 and 3 months of diet feeding schedule (Table 5,6; Fig. 5,6). These findings suggest the development of hypercholesterolemic state, which is in support of extensively reported data in literature (Koul and Kukreja, 1987; Wojcicki et al., 1991; Abraham et al., 1993). In selenium deficient rats, cholesterol and triglyceride levels increased significantly in comparison to adequate selenium fed groups after 1, 2 and 3 months, approximately 62% and 49% increase in cholesterol and triglycerides levels were observed after 3 months in Se deficient control group in comparison to respective adequate group. Also, as the Se deficiency progressed i.e. after 2 and 3 months, the lipid levels increased in comparison to 1-month results in both the selenium deficient groups. Present data is in agreement to the literature that selenium deficiency results in a significant increase in plasma cholesterol levels (Stone et al., 1986; Lee et al., 2003). This is due to increased expression of HMG-CoA reductase (rate limiting enzyme in cholesterol biosynthesis) during selenium deficiency as observed in the present study (Nassir et al., 1997). This increased expression of HMG-CoA reductase during selenium deficiency leads to increased cholesterogenesis in the liver.

This hypercholesterolemic effect of Se deficiency appeared to be potentiated by the addition of cholesterol to the diet, as a 129%, 145% and 159% increase in cholesterol level was observed in high cholesterol diet fed selenium deficient group (Ib) in comparison to respective controls after 1, 2 and 3 months (Stone, 1988; Stone et al., 1994). As mentioned above, low selenium level, which is associated with increased platelet aggregation, leads to cardiovascular complications. Hafeman and Hoekstra (1977) have suggested that selenium
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deficiency promotes *in-vivo* lipid peroxidation, malondialdehyde a chemical by-product of lipid peroxidation modifies the lysine residues on LDL, which results in a decreased degradation of LDL (Haberland *et al*., 1982). Moreover, selenium deficiency leads to hypothyroid state, which is responsible for reduced removal rate of triglyceride from plasma and resulting in its accumulation (Nikkila and Kekki, 1979).

On selenium supplementation (1ppm) in groups IIIa and IIIb, the lipid levels decreased significantly in comparison to respective adequate selenium fed groups. Also in selenium supplemented groups as the time period increased i.e. after 2 and 3 months cholesterol and triglycerides levels decreased in comparison to 1 month treatment period (Table 5,6; Fig. 5,6). This selenium potential against hypercholesterolemia has earlier been reported in our laboratory (Kang *et al*., 2000), as well as by Wojcicki *et al*., (1991). The later have reported an increase in HDL cholesterol fraction on selenium supplementation. HDL cholesterol fraction may down regulate the total cholesterol via reverse cholesterol transport to the liver i.e. HDL fraction increases the cholesterol elimination from tissues including smooth muscle cells in the aorta wall and facilitate the cholesterol transport to the liver, thus preventing its deposition and formation of atheromatous plaque (Marks, 1979). Further, Wojcicki *et al*., (1991) have demonstrated that selenium supplementation normalized the increased MDA concentrations observed in hyperlipidemic rabbits. This suggested that selenium is an effective mean of reducing lipid peroxidation, since MDA is one of the principal products of the breakdown of endoperoxides. Also MDA production leads to TXA2 formation. which is an active vasoconstrictor and platelet-aggregating agent. Wojcicki *et al*., (1991) in their studies have also observed that selenium supplementation lead to elevated levels of cytochrome *P*-450 in the liver microsomes, further cytochrome *P*-450 stimulates 7α-hydroxylase, that converts cholesterol to bile acids and in turn removes it from circulation (Myant *et al*., 1977).
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The effect of selenium supplementation on endocrine functions should also be considered in relation to the mechanism of hypocholesterolemic action of selenium supplementation. Our findings suggest that elevated levels of thyroid hormones are also responsible for normalization of lipid levels in selenium-supplemented animals. Thyroid hormones affect the mechanism of reverse cholesterol transport by influencing the activity of hepatic lipase and CETP, thus modulate the distribution of HDL (Barth et al., 1987) as the elevated level of thyroid hormones result in an increased HDL to LDL ratio (Mullar and Seitz, 1984; Efstathiadou et al., 2001; Ganotakis et al., 2003).

LDL Levels

LDL has long been implicated in the development of atherosclerosis (Mabuchi et al., 2004). In the genetic disorder, familial hypercholesterolemia (FH), the patients develop massive LDL concentrations and frequently die within the second decade of life from complications of coronary artery disease (Fredrickson et al., 1972; Hogue et al., 2004). In the present results on cholesterol supplementation in different groups, the LDL levels increased in comparison to respective control groups after 1, 2 and 3 months of diet feeding schedule. Also in selenium deficient and selenium adequate HCD fed groups, as the cholesterol accumulation increased with time i.e. after 2 and 3 months the LDL levels increased when compared to 1-month results (Table 7; Fig. 7). 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). This increase in the plasma LDL levels on cholesterol supplementation 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. These monocytes adhere to the endothelial cells and subsequently enter into the media, differentiate into macrophages, and eventually become foam cells. These foam cells are
characterized by a massive accumulation of cholesterol esters, (Ross et al., 1993) resulting from the unrestricted uptake of oxLDL.

LDL levels in the serum increased in Se deficiency in comparison to adequate selenium fed groups (IIa and IIb) after 1, 2 and 3 months. Also, in both the Se deficient groups as the time period increased i.e. after 2 and 3 months, the LDL concentration increased (Table 7; Fig. 7). Selenium deficiency leads to enhanced lipid peroxidation that in turn is believed to be involved in oxidative modification of LDL resulting in its reduced clearance through LDL-R. Henrikson et al., (1981) have demonstrated that oxLDL has the ability to induce cholesterol accumulation in macrophages. Studies have shown that oxLDL could stimulate the release of macrophage colony-stimulating factor (M-CSF) (Rajavashisth et al., 1990) and monocyte chemo attractant protein-1 (MCP-1) (Cushing et al., 1990) from endothelial cells. That in turn would facilitate the development of fatty streak lesions by recruiting monocytes and facilitating their differentiation into tissue macrophages. 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 (Takahashi et al., 2005). Circulating oxidized LDL is associated with increased risk of coronary heart disease (Ehara et al., 2001; Holvoet et al., 2001).

Basically in patients with Familial Hypercholesterolemia 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).
Further, on 1ppm selenium supplementation, the LDL levels decreased in comparison to adequate selenium fed animals. In selenium supplemented groups as the time period increased, the LDL levels significantly decreased (Table 7; Fig. 7). Selenium being a potent and well known antioxidant, its supplementation leads to reversal of above said factors i.e. selenium supplementation might be protecting the LDL from oxidative modifications and further atherogenic changes (Hussein et al., 1997; Gonca et al., 2000). Also, upregulation of LDL receptor expression on selenium supplementation might have offered more receptor sites for LDL binding and its clearance from the circulation.

**T₃ and T₄ Levels**

Lipid abnormalities may attribute to the impaired thyroid function (Althaus et al., 1988; Canturk et al., 2003). Prior to the availability of serum thyroid hormone measurements, the serum cholesterol level was used to assist in the diagnosis of hypothyroidism (Bloomer and Kyle, 1959). In the present studies, on high cholesterol diet feeding, T₃ levels decreased and T₄ levels increased in comparison to respective control groups in all the three Se status groups after 1, 2 and 3 months. As cholesterol accumulation increased in HCD fed selenium deficient and adequate groups, the T₃ levels decreased and T₄ levels increased (Table 8,9; Fig. 8,9). So the present study in agreement with the literature clearly indicates that hypercholesterolemia induces hypothyroidism (Glueck et al., 1991; Wojcicki et al., 1991). Sundaram et al., (1997) have shown that LDL is more susceptible to oxidation in patients with hypothyroidism. this oxidized LDL is not taken up by LDL receptors, it accumulates in the body and inturn leads to hypercholesterolemia. So hypothyroidism leads to hypercholesterolemia is also equally true statement (Jung et al., 2003). Our data also supports this i.e. in selenium deficient groups as the T₃ level decreased the lipid levels (total cholesterol and triglycerides) as well as LDL levels increased. It has been suggested in literature that hypothyroid state is responsible for a significantly lower HDL cholesterol fraction, and HDL is responsible for lowering the blood
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cholesterol levels by reverse cholesterol transport from liver (Mullar and Seitz, 1984).

T₃ levels in serum decreased and T₄ levels increased significantly during Se deficiency in comparison to adequate selenium fed groups at all the treatment intervals i.e. after 1, 2 and 3 months of diet feeding schedule. Also as the selenium deficiency progressed in groups Ia and Ib, T₃ levels decreased and T₄ levels increased after 2 and 3 months in comparison to 1-month results (Table 8,9; Fig. 8,9). This is probably due to the decreased conversion of T₄ to T₃ in the liver and the other peripheral tissues due to decreased 5'-DI activity as well as mRNA expression in Se deficiency (Backett et al., 1987; Arthur et al., 1990; Chanoine et al., 1992). Increased plasma T₄ concentration usually gives rise to increased level of hepatic 5'-DI (Berry et al., 1990). In the present results although plasma T₄ level increased in Se deficiency but the hepatic 5'-DI expression was diminished, presumably because the hepatic stores of selenium are insufficient to allow the synthesis of 5'-DI. Further, selenium deficiency could affect the metabolism of thyroid hormones by decreasing glutathione peroxidase activity in the thyroid and thus increasing the concentrations of hydrogen peroxide, in the thyroid. Glutathione peroxidase 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.

T₃ level significantly increased and T₄ level decreased on 1ppm selenium supplementation in groups IIIa and IIIb in comparison to respective adequate groups. In selenium supplemented groups, T₃ level increased and T₄ level decreased significantly after 2 and 3 months of respective diet feeding in comparison to 1-month data (Table 8,9; Fig. 8,9). This could be due to the fact that probably on selenium supplementation, increase in 5'-DI expression upregulated
the T₄ to T₃ conversion. These findings suggest that selenium supplementation up to 1 ppm (level well below the subtoxic level) normalizes the T₃ and T₄ concentrations or regulates the hypothyroidism induced by hypercholesterolemia. Studies have shown that thyroid hormone supplementation can inhibit collagen induced platelet aggregation (Mamiya et al., 1989; Masaki et al., 1992) and can directly relax vascular muscles (Ishikawa et al., 1989). Arem and Patsch (1990) have demonstrated that thyroid hormone replacement therapy in hypercholesterolemia resulted in marked decrease in total cholesterol and LDL cholesterol. Present results are also in agreement to this fact because in group IIIa and IIIib animals, as the T₃ levels increased total cholesterol as well as LDL concentrations decreased significantly.

Basically T₃ is involved at the transcriptional level in cardiovascular system. It enters into the cardiomyocyte through T₃-binding nuclear receptors and interacts with specific transcriptional activators to modify the rate of transcription of specific target genes (Brent, 1994). Thyroid hormones also exert an important effect on the vascular system. It acutely reduces peripheral vascular resistance by promoting relaxation in vascular smooth-muscle cells (Klemperer et al., 1995; Ojamaa et al., 1996a; Park et al., 1997). Napoli et al., (2001) reported that thyroid hormones exert profound effects on vascular reactivity by improving both endothelium-dependent and independent mechanisms.

Bastenie et al., (1972) have shown that thyroid antibodies are much more common in subjects with coronary heart disease than in general population. Pallas et al., (1991) have suggested in their studies that more than 25% of the patients with hypercholesterolemia were positive for thyroid antibodies. Untreated hypothyroidism leads to pathological cardiovascular manifestations such as decreased intravascular volume, increased systemic vascular resistance and hypertension. Diastolic hypertension may be present in approximately 20% of the patients suffering from hypothyroidism (Klein, 1990). The coexistence of
hypertension and lipid disorders in thyroid failure may accelerate the process of atherosclerosis.

**Type-I 5’- Iodothyronine Deiodinase Activity and mRNA Expression**

Type-I 5’-iodothyronine deiodinase (5’-DI) is one of the principal enzymes involved in intrathyroidal and peripheral metabolism of thyroid hormones (Berry et al., 1991b; Geyten et al., 2005) as it converts \( T_4 \) to \( T_3 \). Consistent with the previous work, rats fed with selenium deficient diet had decreased 5’-DI activity as well as mRNA expression in liver and aorta (Beckett et al., 1989; Arthur et al., 1990; Koorle, 1990) in comparison to respective adequate Se diet fed groups. Also, in selenium deficient groups as the deficiency increased i.e. after 2 and 3 months, the 5’-DI activity and expression decreased significantly in comparison to 1-month results (Table 10,11,18,19; Fig. 10,11,29a,29b,30a,30b). Hill et al., (1992) have also reported a decreased 5’-DI mRNA expression in selenium deficiency. This is due to the fact that selenium being the essential structural component of this enzyme, its deficiency leads to decrease in 5’-DI expression. Depalo et al., (1994) concluded that decrease in 5’-DI protein during selenium deficiency was due to a block in the UGA-directed selenocysteine incorporation during translation.

On high cholesterol diet feeding, 5’-DI activity as well as mRNA expression decreased in liver and aorta in comparison to control groups. Also as the cholesterol deposition increased after 2 and 3 months, the 5’-DI expression decreased (Table 10,11,18,19; Fig. 10,11,29a,29b,30a,30b). This could be due to the fact that hypercholesterolemia, which is associated with selenium deficiency, might deplete the selenium pool that is needed for normal 5’-DI expression. Another reason for this could be that hypercholesterolemia as said above, induces hypothyroidism that inturn leads to decreased 5’-DI levels in liver and other peripheral tissues (Emerson et al., 1988; Berry et al., 1990; Santini et al., 1993; Verhoelst et al., 2004). In our results, in selenium deficient groups during hypercholesterolemia, hepatic 5’-DI activity decreased by 40% and mRNA
expression decreased by 33% after 3 months, suggesting that hypercholesterolemia along with selenium deficiency is affecting the 5'-DI enzyme at post-transcriptional level more severely than at transcription level.

Further, in the present results a significant increase in 5'-DI activity as well as mRNA expression in liver and aorta was observed on 1ppm selenium supplementation. Gross et al., (1995) reported a clear dependence of 5'-DI expression on selenium supply in their cell culture experiments in porcine kidney cells, 5'-DI activity as well as mRNA levels rapidly increased with the increase in selenium concentration. Depalo et al., (1994) in SD male rats reported that selenium supplementation to selenium deficient animals resulted in significant increase in 5'-DI activity in liver and kidney. However, Behne et al., (1992) have found that elevated selenium level in the liver after intake of diet having 2ppm Se in the form of selenite was not accompanied by an increase in the 5'-DI activity. Arthur et al. (1990) suggested that in selenium deficient rats, 5'-DI activity was restored on selenium supplementation up to 200μg of Se/kg body-weight/day within 5-8 days. Selenium supplementation to the patients suffering from myxedematous cretinism due to low selenium and iodine intake resulted in a marked drop in serum T4 concentrations through an increase in 5'-DI activity (Contempre et al., 1991).

In contrast to liver and aorta, thyroidal 5'-DI activity and mRNA expression increased significantly in selenium deficiency as well as on high cholesterol diet feeding (Table 12,20; Fig. 12,31a,31b). Increased expression of thyroidal 5'-DI is signaled by higher plasma T4 levels found in selenium deficient rats (Erickson et al., 1982; Arthur et al., 1990). This increased expression of thyroidal 5'-DI in selenium deficiency suggests that thyroid is the higher priority tissue than liver and aorta for selenium when intake of the element is very low, and it is consistent with the fact that thyroid has the ability to retain significant pool of trace element in selenium deficiency. This study is supported by earlier tracer studies of selenium loss from various tissues in the rat during dietary
selenium deficiency, which demonstrated that the thyroid is more efficient than peripheral tissues (liver & aorta) in retaining the trace element (Behne et al., 1988). Zagrodzki et al., (1998) have demonstrated in cattle that in iodine deficiency, a marked 10-12 fold increase in 5’-DI activity was observed in thyroid gland, indicating both tissue specific regulation and adaptation of 5’-DI expression to the physiological demand. The increased T₃ production at the local level and distinct regulation of this individual selenoprotein (5’-DI) in the thyroid is independent of the selenium supply to the body.

This increased 5’-DI activity in thyroid can also be attributed to the compensatory mechanism that might get activated when 5’-DI activity is downregulated in other peripheral tissues. The cellular GSH-Px might represent an intracellular storage form of selenium under selenium deficiency (Evenson et al., 1992). Kohrle (1994) suggested that it was not clear if high level of GSH-Px found in thyroid provided a selenium source for the maintenance of 5’-DI expression.

The requirement to retain selenium within the thyroid and other vital organs probably reflects a crucial importance for selenoproteins within these organs. In the thyroid, GSH-Px and 5’-DI are essential for thyroid hormone production: GSH-Px may also exert a protective function in preventing irreversible oxidative damage to the gland. It has also been hypothesized that selenium deficiency may be responsible, in part for the pathogenesis of endemic myxoedematous cretinism, in that low thyroidal GSH-Px would fail to protect against peroxidative damage to the gland. Due to the limited availability of the tissue, we could not analyze the Se concentrations in the thyroid tissue. However a study by Behne et al., (1988) indicated a very high uptake of ⁷⁵Se in the thyroid of Se deficient rats. They also reported that in Se deficiency there is a strict hierarchy of selenium supply to selenoproteins. Evidence was presented that within a tissue selenium is incorporated into selenoproteins other than GSH-Px. Our data also suggested that in selenium deficiency, hepatic 5’-DI activity decreased by 55%, whereas GSH-Px level decreased by 74% after 3 months of diet feeding schedule. So 5’-DI is
getting the priority over GSH-Px for the trace element during selenium deficiency. Oertal et al., (1993) have also demonstrated a similar hierarchy for selenium supply in cultured porcine kidney epithelial cells with 5′-DI taking priority over GSH-Px.

Arthur et al., (1990) have reported that selenium deficiency leads to elevation of plasma TSH levels that in turn is responsible for increased synthesis and secretion of thyroglobulin by thyroid gland, which further is responsible for iodine deficiency. This iodine deficiency concurrent with the selenium deficiency can exacerbate the hypothyroid state, but thyroidal 5′-DI expression as currently observed which is retained during selenium deficiency plays its role here. As it maintains the T₃ levels in the body, so it has a protective role during selenium deficiency induced hypothyroid stress.

On 1ppm selenium supplementation, 5′-DI activity and expression was further increased in thyroid (Table 12,20; Fig. 12,31a,31b). Beech et al., (1995) demonstrated that human thyrocytes grown in primary culture in a selenium free medium were able to retain the trace element at a level which allowed continued expression of significant activity of 5′-DI. The expression was further increased by addition of selenium.

The changes in plasma thyroid hormone levels occur under various pathological conditions or after food restriction (Kaplan, 1986). But animals in the present study fed on selenium deficient diet, had food ad libitum with the same diet composition (except selenium) as animals in other groups. It is therefore apparent that changes observed in T₃ and T₄ levels in selenium deficient animals through 5′-DI enzyme were a direct result of selenium deficiency and were not due to nonspecific reasons or due to dietary changes.

**LDL Receptor Activity and mRNA Expression**

The LDL receptor (LDL-R) binds to the cholesterol rich LDL and removes it from plasma and thereby regulates the plasma cholesterol level (Brown and Goldstein, 1986). Therefore the LDL receptor has a critical role in the regulation
Discussion of plasma LDL levels by mediating its clearance from circulation (Langer et al., 1972; Bilheimer et al., 1979). Wiseman et al., (1993) reported that magnitude of hypercholesterolemia is associated with the variation in LDL-R gene. Studies by Shepherd and Packard (1984) demonstrated that T₃ enhanced the LDL-R activity. Bakker et al., (1998) have demonstrated that the promoter of LDL-R gene contains a thyroid hormone responsive element (TRE) that could allow T₃ to modulate gene expression of the LDL receptor resulting in an increase of LDL clearance.

In the present studies we have observed that in the selenium deficient groups, the LDL-R activity as well as mRNA expression is downregulated in comparison to the adequate selenium diet fed animals after 2 and 3 months of respective diet feeding schedule. Also, as the selenium deficiency progressed in deficient groups, the LDL-R expression decreased (Table 14,15,16,21; Fig. 18,20, 22.32a,32b). This decrease in LDL-R activity as well as expression could be due the decreased T₃ level, observed in selenium deficiency. Ness et al., (1990) have established that expression of the LDL receptor gene and protein was repressed when thyroid hormone levels were experimentally decreased. Shin and Osborne (2003) have demonstrated that the gene encoding SREBP-2 (sterol regulatory element-binding protein), a major transcriptional regulator of genes involved in cholesterol uptake and synthesis, is directly regulated by thyroid hormones. Also, as we have observed that in selenium deficiency cholesterol level increased, this increased intracellular cholesterol level might have down regulated the LDL-R expression through feedback signaling pathway. Liu et al., (1997) have demonstrated by RT-PCR and Northern blotting that high cholesterol level decreased the LDL-R mRNA level. Goldstein and Brown (1990) suggested that LDL-R expression is regulated predominantly through cholesterol negative feedback pathway. Uptake of plasma LDL cholesterol results in an elevated intracellular cholesterol concentration, which suppresses the transcription of LDL-R in a well characterized example of end product feedback repression.
On feeding high cholesterol diet to the animals, LDL-R activity and mRNA expression decreased in all the three selenium status groups (Table 14,15,16,21; Fig. 19,21,22,32a,32b). So exogenous cholesterol given through diet is being used in the signaling pathway and probably it is suppressing the transcription of LDL-R through feedback suppression. Maximum downregulation in LDL-R activity as well as expression was observed in selenium deficient, cholesterol fed group. Therefore dietary cholesterol is having the additive effect along with the selenium deficiency. Ishibashi et al., (1993) 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.

Supplementation of selenium in the present study resulted in increased LDL-R activity as well as mRNA expression after 2 and 3 months of diet feeding schedule (Table 14,15,16,21; Fig. 18,20,22,32a,32b). Decrease in total cholesterol as well as LDL-cholesterol levels on selenium supplementation in groups IIIa and IIIb might have lead to an increase in LDL-R expression again through feedback inhibition pathway. Moreover, in the present results selenium supplementation lead to normalization of hypothyroidism (T₃ and T₄ levels) through selenium dependent 5'-DI and owing to the dependence of LDL-R expression on T₃ levels, the receptor level may tend to increase in selenium-supplemented animals. 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 defects in the LDL receptor-mediated catabolism of LDL (Abraham et al., 1981). Studies by Walton et al., (1965) in hypothyroid patients, using radiolabelled LDL, demonstrated a prolonged half-life of LDL cholesterol because of decreased catabolism of LDL through LDL-R, an effect that was reversible with thyroid hormone therapy. Moreover, selenium being a potent antioxidant its supplementation leads to the
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prevention of oxidative modification of LDL and faster clearance rate of LDL from the blood and in turn to the enhancement of LDL-R activity.

In the present results, after one month of treatment, no significant change in the LDL-R activity as well as mRNA expression was observed (Table 13, 16, 21: Fig. 16, 17, 22, 32a, 32b). However after 2 and 3 months of diet feeding schedule, as discussed above, significant changes were there. This might be due to the reason that after 1 month the cholesterol accumulation might not be up to the extent that it could stimulate feedback signaling pathway at translational as well as at transcriptional level for LDL-R. However, after 2 and 3 months, there might be sufficient cholesterol accumulation to stimulate the feedback-signaling pathway to downregulate the LDL receptor.

The present study provides the evidence that selenium deficiency leads to the down regulation of LDL-R activity as well as mRNA expression. Further excess of cholesterol in the diet has additive effect on this selenium dependent regulation. Selenium supplementation lead to increase in the clearance of $^{131}$I-LDL from blood. These findings do not suggest that selenium has some direct implication on the LDL-R levels, rather it might be due to the antioxidant role of selenium as well as through selenium dependent behavior of 5'-DI enzyme that in turn regulates the thyroid hormone levels as observed in the present study. However, this interrelationship between selenium status and LDL-R gene expression warrants further investigation to decide the precise mechanism of cholesterol metabolism through the effect of selenium status on the LDL-R gene expression.

These results form the basis for a model to account for hypothyroidism-linked hypercholesterolemia. This model predicts that decrease in 5'-DI and in turn thyroid hormone levels during selenium deficiency as well as on high cholesterol diet feeding followed closely by a drop in LDL receptor mRNA expression. This results in a decline in high affinity LDL cholesterol uptake in the liver resulting in hypercholesterolemia, which is fundamentally linked to thyroid hormone status.
Thyroid hormone depletion not only downregulates liver LDL receptor mRNA and protein, but hypothyroid humans have increased serum LDL cholesterol and reduced LDL uptake that can be reversed by T3 supplementation.

**Apolipoprotein B Expression**

Basically apolipoprotein B (apoB) contains the ligand-binding domain for the binding of LDL to LDL-R site, which enables the removal of LDL from the circulation. So it has a major role in determining plasma cholesterol levels in humans and other mammalian species (Brown and Goldstein, 1985). In the genetic disorder familial defective apoB-100 (FDB), in which high levels of LDL accumulate in the circulation, basically mutations in the apoB disrupt the binding of LDL to its receptor (Innerarity et al., 1990; Kaiser et al., 2002; Dedoussis et al., 2004).

Previous studies in literature have shown that hypercholesterolemic diet feeding lead to upregulation of apoB levels. In the present studies, on high cholesterol diet feeding for 2 and 3 months, apoB levels by ELISA as well as its expression by western blotting increased in all the three groups. Also, in groups Ib and IIb as the cholesterol accumulation increased after 2 and 3 months, the apoB levels increased in comparison to 1-month results (Table 17; Fig. 23,26,27). Abraham et al., (1993) have found a significantly increased level of apoB in cultured hepatocytes isolated from rats fed with atherogenic diet. Kosykh et al., (1988) have observed increased synthesis of apolipoprotein B in cholesterol fed rabbit hepatocytes. Ouguerram et al., (2004) demonstrated that over production of apoB resulted in disturbed cholesterol homeostasis. Kumar et al., (1992) reported that incubation of hepatocytes isolated from normal rats with cholesterol resulted in an increased synthesis and secretion of apoB levels. This increased level of apoB on high cholesterol feeding is due to decreased expression of LDL-R during hypercholesterolemia as observed in the present studies. This decreased level of LDL-R is responsible for decreased clearance of apoB along with LDL, so these
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Apolipoproteins are accumulated in the body (Brown and Goldstein, 1986; Whitfield et al., 2004).

Williams and Tabas (1995) have demonstrated that apoB is known to interact with proteoglycans, a process that is relevant in the pathogenesis of atherosclerosis. Proteoglycans exist on the endothelial cell surface as well as inside the intima. Proteoglycans by binding on endothelium may facilitate lipoprotein entry into the vascular intima and it activates or accelerates plaque progression. So, increased expression of apoB on cholesterol feeding activates the plaque progression.

In the present results in selenium deficiency apoB levels in liver increased significantly in both la and lb groups in comparison to adequate groups after 2 and 3 months. As the Se deficiency increased after 2 and 3 months, the apoB expression increased in comparison to 1-month results. This is consistent with the findings of Mazur et al., (1996), they concluded that rats deficient in selenium and vitamin E had decreased apoB levels. This could be due to the reason that selenium deficiency as already discussed lead to hypothyroid state through decreased expression of 5’-DI enzyme (Verhoelst et al., 2004). Hypothyroidism has been associated with increased level of apoB (Dolphin and Forsyth, 1983). As T3 is directly involved in LDL-R expression, so reduced T3 activity during selenium deficiency leads to increase in apoB levels. Staels et al., (1990) have demonstrated that thyroid hormones activate the LDL-R, leading to an increased fractional catabolic rate of apoB without influencing its synthesis rate. Davidson et al., (1990) have found that T3 administered to hypothyroid animals reduce plasma apoB concentrations.

In hypothyroid subjects, decreased activity of the LDL receptor has been observed and it is shown to be reversible after thyroid hormone replacement (Walton et al., 1965; Chait et al., 1979; Thompson et al., 1981). ApoB synthesis increased in hypothyroid animals compared to that in hyperthyroid animals (Davidson et al., 1990). De-Brun et al., (1993) and Ganotakis et al., (2003)
observed that decrease in apoB concentrations in the treated hypothyroid subjects had been caused by a reduction in plasma LDL and VLDL levels.

In the present studies on 1ppm selenium supplementation for 2 and 3 months decreased expression of apoB was observed. This could be due to the reason that as selenium supplementation leads to reversal of hypothyroidism and hypercholesterolemia, so it results in increased catabolic rate of apoB through increased LDL-R expression. Davidson et al., (1988) have demonstrated the suppressed synthesis of apoB during T₃ supplementation. Walton et al., (1965) have suggested the increased catabolism of apoB through LDL receptors during hyperthyroidism. It is probably a combination of suppressed apoB synthesis and its increased elimination via LDL receptors during selenium supplementation here, which is regulating the apoB expression through T₃ and inturn by type-I 5'-iodothyronine deiodinase enzyme.

After 1 month of treatment no change was observed in apoB levels. This could be due to the fact that after 1 month as observed in the present studies. LDL-R activity as well expression was not altered. So apoB catabolism through LDL receptors was not affected in different groups.

**HMG-CoA Reductase mRNA Expression**

HMG-CoA reductase catalyzes the rate-limiting step in cholesterol biosynthesis. It also has a significant role in cholesterol homeostasis (Ness and Chambers, 2000). Basically this enzyme is the target for the statin class of cholesterol-lowering drugs that are currently being used so effectively.

In the present studies, the HMG-CoA reductase mRNA expression decreased in all the three selenium status groups on cholesterol feeding. This is in agreement with the findings in the literature by Ness and Gertz (2004) that hepatic HMG-CoA reductase expression is markedly downregulated when animals face a cholesterol challenge with high dietary cholesterol (Table 22; Fig. 33a,33b). To support this, Ness and Chambers (2000) have demonstrated that when given high cholesterol diet, the dietary cholesterol suppresses the endogenous cholesterol
production by inhibiting the expression of hepatic HMG-CoA reductase to compensate for increased absorption of dietary cholesterol. So the present studies in agreement to the literature suggest that HMG-CoA reductase expression is downregulated at transcriptional level to compensate for the high dietary cholesterol levels when fed with HCD to enable the body to regulate its own cholesterol content by a local feedback control system. Brown et al., (1974) have suggested that LDL and apoB levels in the body have additive effect along with the cholesterol to regulate the HMG-CoA reductase expression. In the present study as we have observed increased level of LDL and apoB in high cholesterol fed groups, this could be another reason for HMG-CoA reductase downregulation.

Feedback regulation of hepatic cholesterol biosynthesis was recognized nearly 50 years ago (Gould and Taylor, 1950). Over the intervening years, several mechanisms for this feedback regulation of hepatic HMG-CoA reductase in response to dietary cholesterol have been postulated. The mechanisms that are currently being examined include: transcription, translation, protein turnover, and regulation of catalytic efficiency by phosphorylation/dephosphorylation (Goldstein and Brown, 1990).

Studies in rats, hamsters and mice have revealed apparent transcriptional regulation (Liscum et al., 1983). Feeding mice with the diets containing up to 5% cholesterol for 10 days caused 3–4-fold decrease in hepatic reductase mRNA levels when compared with animals that received diets supplemented with only corn oil (Rudling, 1992). In experiments with Syrian hamsters, feeding diets containing 2% cholesterol for 14 days reduced the hepatic HMG-CoA reductase mRNA levels by about 6-fold (Gil et al., 1986). In another study in Golden Syrian hamsters, feeding diets containing 0.5% cholesterol for 12 days downregulated hepatic reductase mRNA levels by 3-fold (Shimomura et al., 1997). Shimano et al., (1996) have suggested that the possible mechanism for this transcriptional regulation of HMG-CoA reductase could be the regulation of sterol response
element binding proteins and several other enzymes involved in cholesterol and fatty acid biosynthesis.

Ness et al., (1994) have demonstrated a much greater decline in hepatic HMG-CoA reductase protein than mRNA levels in rats. This study has shown that translation is also the important site for feedback regulation by dietary cholesterol. Further, Chambers and Ness (1998) by pulse labeling studies of HMG-CoA reductase in liver slices to measure the rate of synthesis of reductase protein found that feeding the diets containing 2% cholesterol to rats for 48 hrs. caused a 6-fold decrease in the rate of synthesis of reductase protein. Thus, translation also appeared to be the major mechanism of feedback regulation of hepatic HMG-CoA reductase in rats in response to both exogenous and endogenous cholesterol.

In the present studies in selenium deficient diet fed groups, the HMG-CoA reductase mRNA expression increased (Table 22; Fig. 33a,33b). This was in agreement to the findings of Nassir et al., (1997), they observed increased activity of HMG-CoA reductase in selenium deficient rats. Further it was suggested that basically this was the major significant mechanism through which selenium deficiency lead to hypercholesterolemia. On selenium supplementation (1ppm) significant decrease in mRNA expression was observed in comparison to respective adequate diet fed groups. This could be due the increased T₃ levels on selenium supplementation. As T₃ is responsible for decreased expression of HMG-CoA reductase. Ness et al., (1998) have demonstrated that T₃ supplementation reduced the HMG-CoA reductase enzyme expression.

The relative level of hepatic HMG-CoA reductase gene expression may in part determine an animal's degree of susceptibility to cholesterol and to counteract the effect of dietary cholesterol on serum and tissue levels. Several important areas may prove fertile for future investigations. The molecular mechanism involved in regulation of hepatic HMG-CoA reductase gene expression through the involvement of enzyme 5'-DI via. T₃ and T₄ levels need to be elucidated. Answers to these questions should provide a better understanding of the physiological
regulation of hepatic HMG-CoA reductase and in turn of cholesterol homeostasis through thyroid hormones. With such knowledge, better control of serum cholesterol levels, a major risk factor for atherosclerotic vascular disease, can be attained.

So the present study suggests that hypercholesterolemia and cardiovascular disorders are associated with selenium status through selenoenzyme, 5'-DI. Hypercholesterolemia along with selenium deficiency probably is co responsible for tissue specific differential expression of 5'-DI. Selenium deficiency leads to decreased expression of 5'-DI, increase in the cholesterogenesis in liver and decrease in cholesterol removal from blood through upregulation of HMG-CoA reductase and downregulation of LDL-R activity and mRNA expression respectively. Whereas Se supplementation upto 1ppm leads to increase in the LDL-R activity as well as mRNA expression and in turn has the protective role against hypercholesterolemia. Also it normalizes the $T_3/T_4$ concentrations or regulates the hypothyroidism induced by hypercholesterolemia through its dependent enzyme, 5'-DI.