SUMMARY

Cholesterol homeostasis in the body is maintained in a well-characterized manner and mainly two membrane proteins are considered responsible for this. One is LDL-R that takes up cholesterol from the circulation in the form of LDL and VLDL, so responsible for extracellular cholesterol metabolism. Another parameter that is responsible for intracellular cholesterol homeostasis is HMG-CoA reductase. This enzyme basically is responsible for cholesterol biosynthesis in the body. Both the pathways are regulated through end product feedback mechanism depending upon the need of the body for cholesterol. Extra cellular cholesterol transport is largely mediated by plasma lipoproteins. Excess of cholesterol in the body is responsible for hypercholesterolemia, which is a recognized risk factor for atherosclerosis. A dangerously high level of LDL in blood resulting from failure to produce enough LDL-R causes much of atherogenecity in general population. The LDL receptor mediates the removal of LDL and remnant lipoproteins from circulation by binding to apolipoprotein B.

Involvement of thyroid hormones (T3/T4) have also been reported in context to cardiovascular disorders. Hypothyroidism is said to be associated with hypercholesterolemia and in turn atherosclerosis. Basically T3 is the active form of thyroid hormones and is involved in regulation of LDL-R gene, which is produced from T4 by 5'-monodeiodination. This reaction is catalyzed by 5'-DI enzyme. Selenium being the integral part of this enzyme, the activity as well as expression changes along with the selenium status. Thus selenium is directly regulating the T3 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.

Considering the above factors, aim of the present study was to establish the role of selenium status in modulation of 5'-DI and LDL-R expression along with the associated parameters during experimental hypercholesterolemia.

In the present study significantly decreased levels of selenium were observed in liver and serum in selenium deficient groups. Moreover decreased
level of glutathione peroxidase (GSH-Px) observed during selenium deficiency in the present study confirms the selenium deficient state in the body. Low selenium levels are associated with increased platelet aggregation and thromboxane A2 production along with decreased prostacyclin production. All of these may lead to cardiovascular complications. In the present findings in selenium deficient groups as well as on high cholesterol diet feeding, the cholesterol, triglycerides and LDL levels increased significantly after 1, 2 and 3 months. This strengthens the fact that selenium deficiency is associated with hypercholesterolemia. This can also be attributed to the increased expression of HMG-CoA reductase during selenium deficiency as observed in the present study. This increased expression of HMG-CoA reductase during selenium deficiency might be leading to increased cholesterogenesis in the liver. Basically, selenium deficiency promotes in-vivo lipid peroxidation. Which results in a decreased catabolism of LDL through LDL-R and it is accumulated in the body. Moreover, selenium deficiency leads to hypothyroid state, which is responsible for reduced removal rate of triglyceride from plasma and resulting in its accumulation.

This increase in the plasma LDL levels on cholesterol supplementation and during selenium deficiency lead to its accumulation in the intima, where it is oxidized. This oxidized LDL activates the endothelial cells lining the vessel wall, attracting monocytes from 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.

On 1ppm selenium supplementation, the decrease in cholesterol, triglycerides and LDL levels at all the intervals i.e. after 1, 2 and 3 months might be due to enhanced mobilization of lipids from circulation. Selenium being a potent antioxidant might be protecting the LDL from oxidative modification and leading to its increased binding to the LDL-R. Moreover, the selenium
supplementation basically leads to an increase in HDL cholesterol fraction, which down regulates the total cholesterol via reverse cholesterol transport to the liver from tissues including smooth muscle cells in the aorta wall. Thus preventing its deposition and formation of atheromatous plaque.

Type-I 5′-iodothyronine deiodinase being a selenoprotein, its activity as well as mRNA expression decreased in selenium deficient groups after 1, 2 and 3 months in liver and aorta. On high cholesterol diet feeding also 5′-DI expression decreased significantly in liver and aorta, this might be due to the selenium deficient state created by hypercholesterolemia. It is suggested that 5′-DI expression depends upon selenium supply. It rapidly increases with the increase in selenium concentration. In the present study also, 5′-DI activity as well as mRNA expression increased on 1ppm selenium supplementation.

Significantly decreased level of T₃ and increased level of T₄ observed in the present study during selenium deficiency and on HCD feeding after 1, 2 and 3 months might be due to the decreased 5′-DI levels. Decreased level of 5′-DI enzyme during selenium deficiency as well as on high cholesterol diet feeding might be leading to lesser conversion of T₄ to T₃. So, present study proved that hypercholesterolemia leads to hypothyroidism. On 1ppm selenium supplementation increased T₃ and decreased level of T₄ could be due to the increased expression of 5′-DI. So, the present study suggested that selenium supplementation up to 1 ppm regulates the hypothyroidism induced by hypercholesterolemia through its dependent enzyme 5′-DI. Thyroid hormone normalization on selenium supplementation can inhibit collagen induced platelet aggregation and can directly relax vascular muscles. In the present results in selenium supplemented groups as the T₃ level increased, the lipid levels i.e. cholesterol, triglycerides and LDL levels decreased. This proves that thyroid hormone replacement therapy in hypercholesterolemia showed marked decrease in total cholesterol and LDL levels.
Another interesting outcome of this study was the increased activity as well as mRNA expression of 5′-DI in thyroid in selenium deficiency as well as on high cholesterol diet feeding. This suggested that thyroid is the higher priority tissue than liver and aorta for selenium when intake of the element is very low. It is consistent with the fact that thyroid has the ability to retain significant pool of trace element in selenium deficiency. These findings indicated both tissue specific regulation and adaptation of 5′-DI expression to the physiological demand. In the thyroid, 5′-DI is essential for thyroid hormone production. The increased T3 production at the local level and distinct regulation of this individual selenoprotein in the thyroid seemed to be independent of the selenium supply to the body. So there is some compensatory mechanism that might get activated when 5′-DI expression is downregulated in other peripheral tissues to meet the requirements of body for this important enzyme. This process regulates the level of physiologically important thyroid hormones. Thyroid hormones are said to be controlling the body’s total homeostasis.

In the present studies downregulation of LDL-R activity as well as mRNA expression in both the selenium deficient groups after 2 and 3 months might be due to decreased T3 level. Studies have suggested that a major transcriptional regulator of genes involved in cholesterol uptake through LDL-R is directly regulated by thyroid hormones. Another reason for this downregulation of LDL-R expression might be that increased intracellular cholesterol level during selenium deficiency might have down regulated the LDL-R expression through negative feedback signaling pathway at transcriptional level.

On feeding high cholesterol diet to the animals, the decrease in LDL-R activity and mRNA expression after 2 and 3 months suggested that exogenous cholesterol given through diet was being used in the signaling pathway and probably suppressing the transcription of LDL-R through feedback inhibition. Maximum downregulation in LDL-R activity as well as expression was observed in selenium deficient, cholesterol fed group, so dietary cholesterol had additive
Summary

effect along with the selenium deficiency. Selenium supplementation upto 1ppm in the present study resulted in increased LDL-R activity as well as mRNA expression after 2 and 3 months of diet feeding. This could be due the fact that decrease in total cholesterol as well as LDL-cholesterol levels on selenium supplementation might have lead to an increase in LDL-R expression again through feed back pathway. Moreover, increased level of T3 on selenium supplementation lead to normalization of hypothyroidism 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. The elevated LDL cholesterol levels in hypothyroidism may occur as a result of defects in the LDL receptor-mediated catabolism of LDL. Moreover, selenium being a potent antioxidant its supplementation leads to the 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.

However, after one month of treatment, no significant change in the LDL-R activity as well as mRNA expression was observed. 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 down regulate the LDL receptor expression.

In the present studies on selenium deficient diet feeding as well as on 2% cholesterol supplemented diet feeding for 2 and 3 months apoB expression increased significantly. Basically, decreased expression of LDL-R during hypercholesterolemia as well as selenium deficiency was responsible for decreased clearance of apoB along with LDL. So these apolipoproteins might get accumulated in the body. Another reason for this could be decreased expression of 5′-DI, which is responsible for hypothyroid state. ApoB synthesis is increased in hypothyroid subjects. Further apoB is known to interact with proteoglycans that
are involved in the pathogenesis of atherosclerosis. Proteoglycans may facilitate lipoprotein entry into the vascular intima and accelerate plaque progression by binding to the endothelial cell surface. So, increased expression of apoB on cholesterol feeding activates the plaque progression. On the other hand, the decreased expression of apoB on selenium supplementation (1ppm) in the present studies could be due to the reason that selenium supplementation leads to reversal of hypothyroidism and hypercholesterolaemia. So it resulted in increased catabolic rate of apoB through increased LDL-R expression.

After 1 month, 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 as expression was not altered, so apoB catabolism through LDL receptors was not affected in different groups.

HMG-CoA reductase is involved in cholesterol biosynthesis. Decreased expression of this enzyme on high cholesterol diet feeding was observed. It suggested that high dietary cholesterol suppressed the endogenous cholesterol production by inhibiting the expression of hepatic HMG-CoA reductase to compensate for increased absorption of dietary cholesterol. In the present studies in selenium deficient groups the HMG-CoA reductase mRNA expression increased. Basically this was the major significant mechanism through which selenium deficiency leads to hypercholesterolemia. On selenium supplementation (1ppm) decreased mRNA expression of HMG-CoA reductase was observed. This could probably be due to the increased T3 levels on selenium supplementation. It has been suggested in literature that increased level of thyroid hormones is responsible for decreased expression of HMG-CoA reductase.

In conclusion, the present study indicates that the decreased expression of selenium dependent 5'-DI enzyme could be the key factor behind the occurrence of hypothyroid state during hypercholesterolemia and selenium deficiency. Furthermore, 5'-DI enzyme might be regulating the LDL-R expression through thyroid hormone levels. Whereas Se supplementation up to 1ppm normalized the
T₃/T₄ concentrations or regulated the hypothyroidism induced by hypercholesterolemia through its dependent enzyme, 5'-DI. Also, it lead to increase in the LDL-R activity as well as mRNA expression and in turn had the protective role against hypercholesterolemia. However, this interrelationship warrants further investigation to decide the precise mechanism of cholesterol metabolism through the effect of selenium dependent 5'-DI on the LDL-R gene expression. Until now, no direct association between 5'-DI behavior and hypercholesterolemia has been observed. Thus, longitudinal studies to clarify the potential association between 5'-DI expression and hypercholesterolemia under different Se status must be performed. Further studies must be undertaken to explore the therapeutic role of selenium supplementation in hypercholesterolemia through its dependent enzyme 5'-DI.