Protective role of selenium status on $T_3/T_4$ kinetics in rats under hyperlipidemia

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The effect of high fat diet (HFD) on thyroid hormones ($T_3/T_4$) and protective role of selenium (Se) were studied in rats. Se levels in serum and liver decreased significantly, whereas glutathione peroxidase (GSH-Px) in liver and lipid levels (cholesterol and triglycerides) in serum increased after 1, 2 and 3 months of HFD feeding in comparison to controls in all the three Se status i.e. deficient (0.02 ppm), adequate (0.2 ppm) and excess (1 ppm) groups. Levels of $T_3/T_4$ decreased significantly on HFD feeding, as compared to respective controls in all the groups. Within the deficient group, as Se deficiency progressed, $T_3/T_4$ levels decreased after 2 and 3 months in comparison to 1 month. A significant increase was observed in $T_3/T_4$ concentration on feeding 1 ppm (excess) Se supplemented diet, in comparison to adequate group. Also, in 1 ppm Se supplemented group as the Se deposition increased i.e. after 2 and 3 months, levels of $T_3/T_4$ increased significantly. So, the present study indicates that Se supplementation up to 1 ppm normalizes the $T_3$ and $T_4$ concentrations or regulates the hypothyroidism induced by hyperlipidemia.

Key words: hyperlipidemia, hypothyroidism, lipid, $T_3/T_4$ kinetics, selenium status, rats

Hypercholesterolemia is an accepted risk factor for development of atherosclerosis. Regular intake of high fat diet (HFD), may cause hypercholesterolemia, thus lead to cholesterol deposition in arterial wall. Selenium plays a protective role against HFD-induced alterations through its antioxidant action. Se as an antioxidant has been used experimentally for trials against atherogenesis induced by HFD feeding. Thyroid hormone status has also been shown to have a relationship with atherosclerotic disorders. Changes in thyroid hormone status alter the lipid metabolism and thyroid hormones are suggested to have a regulatory role in the catabolism of high and low density lipoproteins. Hypothyroidism is a possible risk factor for atherosclerosis. It has been reported that normalization of the thyroid hormones with thyroxine administration is accompanied by improvement of lipid abnormalities in the group with sub clinical hypothyroidism. In view of the above findings, present study is focused on the protective role of the Se status in regulation of thyroid hormone levels under normal and hypercholesterolemic conditions.

Materials and Methods
Male Sprague-Dawley rats, (approx. 100 g body wt.) obtained from the Central Animal House, Panjab University, Chandigarh were used in the study.

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Animals acclimatized to the laboratory animal room were divided into three groups. Group I (Se deficient diet fed), group II (Se adequate diet fed) and group III (Se excess diet fed). Feed and water were given ad libitum. This Se diet was given to the animals for 10 days, so as to achieve the required Se status. After ten days, the animals in these three groups were further divided into two each viz.: Group Ia (Se deficient control); Group Ib (Se deficient + HFD); Group IIa (Se adequate control); Group IIb (Se adequate + HFD); Group IIIa (Se excess control); and Group IIIb (Se excess +HFD).

All the three control groups received the respective basic Se status diet, whereas the HFD groups were fed with the respective Se status diet having 2% cholesterol for three months. Weekly monitoring of body weight was done, so as to check the growth of the animals.

Yeast based synthetic Se deficient (supposed to contain 0.02 ppm Se) diet was prepared in the laboratory, as given by Burk. It contains torula yeast (inactivated) 30%, sucrose 56.99%, corn oil 6.67%, mineral mix 5%, vitamin mix 1%, dl-methionine 0.3% and vitamin E 0.04%.

Se adequate and Se excess diet was prepared from Se deficient diet by supplementing it with 0.2 ppm and 1 ppm, respectively of Se as sodium selenite (Sigma Aldrich Co., USA). Cholesterol 2% (Loba-Chemie, India) was added to the respective diets of HFD groups.
After completion of diet feeding schedule i.e. after 1, 2 and 3 months, blood was drawn from the retro orbital sinus of the ether anaesthetized overnight fasting rats. Serum was prepared from the blood. After bleeding, the animals were sacrificed under ether anaesthesia, followed by cervical dislocation. Liver was removed immediately, washed with buffer and stored at –20°C for further analysis as detailed below.

Selenium level was estimated by following the method as described\(^9\) based on the principle that Se content in serum or tissue on acid digestion is converted to selenious acid. The reaction between selenious acid and aromatic-\(\alpha\)-diamines such as 2,3-diamino-naphthalene (DAN) leads to the formation of 4, 5-benzopiazselenol, which displays brilliant lime-green fluorescence, when excited at 366 nm in cyclohexane. Fluorescence emission in extracted cyclohexane was read on fluorescence spectrophotometer using 366 nm as excitation wavelength and 520 nm as emission wavelength.

Serum total cholesterol was estimated by enzymatic colorimetric test kit obtained from Human Diagnostic (Germany) based on CHOD-PAP method. Serum triglycerides level was estimated by enzymatic test kit obtained from Accurex Biomedical.

Activity of glutathione peroxidase (GSH-Px) activity was assayed by the coupled enzyme procedure with glutathione reductase using \(\text{H}_2\text{O}_2\) as substrate\(^10\). The assay was carried out in the post-mitochondrial fraction (PMF) of liver, and the activity expressed as \(\mu\)moles of NADPH oxidized/min/mg protein.

\(\text{T}_3\) and \(\text{T}_4\) levels were estimated in serum by standard Gamma Coat\(\text{TM}\) \([^{125}\text{I}]\) RIA kit obtained from Diasorin (U.S.A.).

Protein estimation was done by using the method of Lowry et al\(^11\).

Data is expressed as mean ± SEM. Difference between different groups was tested using student’s \(t\) test for unpaired values.

### Results

#### Selenium levels

Se levels in the serum and liver decreased significantly \((p<0.001)\) in HFD fed groups as compared to respective controls. Significant decrease/increase in Se level was observed after 2 and 3 months of respective diet feeding in comparison to 1 month data (Fig. 1A and 1B).

#### GSH-Px activity

GSH-Px activity was measured in liver. The activity increased significantly \((p<0.001)\) on HFD feeding, in comparison to respective controls. Also, in all the groups except Ia, it increased significantly after 2 and 3 months as compared to 1 month (Fig. 1C).
Levels of T3 and T4 decreased significantly and present results are also in agreement with this fact. In the present study, Se levels in the serum and liver were estimated in all the treatment groups. Significantly decreased levels of Se were observed in HFD fed groups, as compared to control (Fig. 1A and 1B). These results are in agreement with the findings of Oster and Prellwitz\(^{13}\). Earlier, Subramanayam et al.\(^{14}\) have reported a decrease in the serum Se levels on high cholesterol diet feeding. Low serum Se level is associated with increased platelets aggregation, higher production of thromboxane-A\(_2\) and decreased productivity of prostacyclin, all of which may be linked to the development of cardiovascular diseases\(^{15,16}\).

In the present studies, serum cholesterol and triglycerides level increased significantly in HFD fed groups, as compared to control groups in all the three Se status i.e. deficient, adequate and excess groups (Table 1). Also, if we compare lipid levels in group IIb (Se adequate) with group Ib (Se deficient), there is a significant increase in cholesterol and triglycerides levels in Se deficient group in all the 1, 2 and 3 months diet fed animals, whereas the lipid levels significantly decreased in group IIIb i.e. 1 ppm Se fed group and it decreased significantly in 1 ppm Se supplemented HFD fed group in comparison to adequate HFD fed group. Also, respective increase/decrease in these two parameters was observed after 2 and 3 months in comparison to 1 month data depending upon the Se status (Table 1).

### Table 1—Total cholesterol, triglyceride, T\(_3\) and T\(_4\) levels in serum after 1, 2 and 3 months of HFD feeding schedule

<table>
<thead>
<tr>
<th></th>
<th>Se deficient</th>
<th>Se adequate</th>
<th>Se excess</th>
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<tbody>
<tr>
<td><strong>Cholesterol (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 month</td>
<td>101.5±0.9</td>
<td>252.3±1.4*</td>
<td>91.1±0.5</td>
</tr>
<tr>
<td>2 month</td>
<td>104.5±0.5*</td>
<td>287.6±1.0*</td>
<td>86.1±0.5*</td>
</tr>
<tr>
<td>3 month</td>
<td>110.5±0.7*</td>
<td>327.3±0.62*</td>
<td>82.7±0.5*</td>
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<tr>
<td><strong>Triglycerides (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 month</td>
<td>100.9±0.4</td>
<td>222.3±0.8*</td>
<td>93.6±0.8</td>
</tr>
<tr>
<td>2 month</td>
<td>101.7±0.7</td>
<td>268.9±0.6*</td>
<td>88.9±0.5*</td>
</tr>
<tr>
<td>3 month</td>
<td>105.3±0.3*</td>
<td>310.6±0.5*</td>
<td>82.4±0.5*</td>
</tr>
<tr>
<td><strong>T(_3) (nmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 month</td>
<td>1.16±0.01</td>
<td>0.80±0.01*</td>
<td>1.29±0.01</td>
</tr>
<tr>
<td>2 month</td>
<td>1.02±0.01*</td>
<td>0.66±0.01*</td>
<td>1.62±0.02*</td>
</tr>
<tr>
<td>3 month</td>
<td>0.92±0.004*</td>
<td>0.47±0.007*</td>
<td>1.92±0.003*</td>
</tr>
<tr>
<td><strong>T(_4) (nmol/L)</strong></td>
<td></td>
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</tr>
<tr>
<td>1 month</td>
<td>98.6±0.8</td>
<td>60.9±0.6*</td>
<td>114.3±0.9</td>
</tr>
<tr>
<td>2 month</td>
<td>91.5±0.6*</td>
<td>56.7±0.4*</td>
<td>128.6±0.6*</td>
</tr>
<tr>
<td>3 month</td>
<td>81.6±0.6*</td>
<td>43.5±0.9*</td>
<td>152.5±0.9*</td>
</tr>
</tbody>
</table>

*\(p<0.001\) represents comparison between control and HFD groups; *\(p<0.05\), **\(p<0.01\), ***\(p<0.001\) comparison between 1 and 2 months; *\(p<0.001\) comparison between 1 and 3 months; **\(p<0.001\) comparison between group Ib and IIb; *\(p<0.001\) comparison between group IIb and IIIb.

### arb

**Serum lipid profile**

Rats fed on HFD in different groups i.e. Ib, IIb and IIIb showed a significant increase \((p<0.001)\) in the concentration of cholesterol and triglycerides, compared to the respective control groups. Lipid level increased significantly \((p<0.001)\) in Se deficient HFD fed group and it increased significantly in 1 ppm Se supplemented HFD fed group in comparison to adequate HFD fed group. Also, respective increase/decrease in these two parameters was observed after 2 and 3 months in comparison to 1 month data depending upon the Se status (Table 1).

#### Serum T\(_3\) and T\(_4\) levels

Levels of T\(_3\) and T\(_4\) decreased significantly \((p<0.001)\) on HFD feeding in comparison to respective controls in all the groups. In Se deficiency i.e. in group Ia and Ib values of T3 and T\(_4\) decreased and in adequate and 1 ppm Se supplemented groups, it increased significantly after 2 and 3 months in comparison to 1 month data (Table 1).

### Discussion

Hypercholesterolemia and cardiovascular disorders have been shown to be associated with Se deficiency\(^{12}\) and present results are also in agreement with this fact. In the present study, Se levels in the serum and liver were estimated in all the treatment groups. Significantly decreased levels of Se were observed in HFD fed groups, as compared to control (Fig. 1A and 1B). These results are in agreement with the findings of Oster and Prellwitz\(^{13}\). Earlier, Subramanayam et al.\(^{14}\) have reported a decrease in the serum Se levels on high cholesterol diet feeding. Low serum Se level is associated with increased platelets aggregation, higher production of thromboxane-A\(_2\) and decreased productivity of prostacyclin, all of which may be linked to the development of cardiovascular diseases\(^{15,16}\).

In the present studies, serum cholesterol and triglycerides level increased significantly in HFD fed groups, as compared to control groups in all the three Se status i.e. deficient, adequate and excess groups (Table 1). Also, if we compare lipid levels in group IIb (Se adequate) with group Ib (Se deficient), there is a significant increase in cholesterol and triglycerides levels in Se deficient group in all the 1, 2 and 3 months diet fed animals, whereas the lipid levels significantly decreased in group IIIb i.e. 1 ppm Se fed group and it decreased significantly in 1 ppm Se supplemented HFD fed group in comparison to adequate HFD fed group. Also, respective increase/decrease in these two parameters was observed after 2 and 3 months in comparison to 1 month data depending upon the Se status (Table 1).
supplemented group after 1, 2 and 3 months of diet feeding. Within group Ib, as the Se deficiency progressed i.e. after 2 and 3 months, the cholesterol and triglycerides levels increased significantly in comparison to 1 month data, whereas in HFD fed 1 ppm Se supplemented group as the Se deposition increased i.e. after 2 and 3 months, the cholesterol and triglycerides levels decreased significantly in comparison to 1 month data. This Se potential against hyperlipidemia has earlier been reported in our laboratory, as well as by Wojcicki et al. The later have reported an increase in HDL cholesterol fraction on Se supplementation, HDL cholesterol fraction may depress 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.

It can be interpreted from our results that within Se deficient group Ia, along with the Se status i.e. after 2 and 3 months the level of GSH-Px decreased in comparison to 1 month data. Whereas in group Ib (Se deficient, HFD fed), after 2 and 3 months, level of GSH-Px increased significantly in comparison to 1 month data, also in adequate and 1 ppm Se supplemented groups in control as well as in HFD fed animals both, the level of GSH-Px increased after 2 and 3 months in comparison to 1 month data (Fig. 1C). This increase in Se dependent GSH-Px on HFD feeding is attributed to the increased lipoperoxidative stress associated with HFD 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. This increase in the tissue Se-dependent GSH-Px on HFD feeding explains the decrease in the Se levels as observed in the present study, even though diet contains adequate as well as excess level of Se in the groups IIb and IIIb respectively.

Lipid abnormalities may attribute to the impaired thyroid function. Also, prior to the availability of serum thyroid hormone measurements, the serum cholesterol level was used to assist in the diagnosis of hypothyroidism. Present results showed a significant decrease in the levels of T3 and T4 on HFD feeding. In all the Se status groups, there is a significant decrease in the thyroid hormones on HFD feeding in comparison to control groups after 1, 2 and 3 months of respective diet feeding (Table 1), so the present study in agreement with the literature clearly indicates that hypercholesterolemia induces hypothyroidism. In Se deficient HFD fed group i.e. Ib as Se deficiency progressed after 2 and 3 months, values of T3 and T4 decreased significantly, whereas in adequate and 1 ppm supplemented groups after 2 and 3 months hormone levels increased significantly in comparison to 1 month data. Also, if we compare HFD fed animals of adequate Se diet group with Se deficient group, at every stage of T3 and T4 measurement i.e. after 1, 2 and 3 months, the level decreased significantly in Se deficiency. Whereas on supplementation of 1 ppm Se along with HFD T3 and T4 levels increased significantly, 33% increase in T3 and 21% increase in T4 is observed after 3 months of 1 ppm Se supplementation.

These findings suggest that Se supplementation up to 1 ppm (level well below the subtoxic level) normalizes the T3 and T4 concentrations or regulates the hypothyroidism induced by hyperlipidemia. With this kinetic study, we would like to draw the attention to Se supplementation as a new etiology of hypothyroidism under hyperlipidemia.

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