Hyperlipidemia and Type-I-5'-monodeiodinase activity: Regulation by selenium supplementation

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Received 23 July 1999; revised 15 November 1999

Effect of feeding high fat diet (HFD) and selenium supplemented high fat diet for three months on Type-I 5'-monodeiodinase (5'-DI) activity in rats was studied. There was significant increase in serum cholesterol and triglyceride levels in HFD fed group as compared to the control and when compared to that of Se supplemented group. HFD feeding resulted in decreased serum Se levels but supplementation of Se with HFD resulted in increase in serum Se. Se-dependent glutathione peroxidase (GSH-Px) activity in liver and aorta increased significantly in HFD fed animals and showed additional significant increase on Se supplementation. In HFD fed group, decrease in the low density lipoprotein (LDL) receptor levels was found whereas in the Se-supplemented HFD group the LDL levels were normal. Both serum T3 and T4 levels decreased significantly on HFD feeding. However, on supplementation of Se to HFD, significant increase in T3 and T4 was observed. The activity of 5'-DI in liver decreased significantly in HFD group, but increased in both liver and aorta in Se supplemented HFD group whereas the reverse was true in thyroid.

Materials and Methods

Materials

Cholesterol was obtained from LOBA Chemicals, Mumbai (India). Sodium selenite was from Sigma Chemical Co. (USA). All other reagents and chemicals were obtained from SISCO Research Laboratories, India. High specific activity Na-215I (100 mCi/ml) was obtained from Bhabha Atomic Research Centre, Mumbai, India.

Treatment protocol

Thirty male Sprague-Dawley rats (100-125 g) were obtained from the Central Animal House of the Panjab University, acclimatized to laboratory animal room and divided into three equal groups (10 animals in each): group I, control; group II, high fat diet (HFD) fed and group III, HFD fed+Se supplemented. Water was given ad libitum. Treatment protocol was for three months.

Synthetic diet was prepared in the laboratory according to the composition of Abraham et al.8 The detailed diet composition is as used in our recently published study9. HFD constituted 2% cholesterol, 25 mg/100 g body wt/day or potassium perchlorate and 0.5% sodium cholate along with other essential diet components viz. vitamins and salts, whereas control diet was prepared without above said constituents but containing all the essentials to make it a balanced control diet. The Se supplemented group of animals received 25 μg Se (equivalent to 1.0 ppm in diet) as sodium selenite/rat/day in solution form by oral intubation. It is well established that levels exceeding 0.02 ppm are adequate. Se levels in mammals, and levels beyond 2.0 ppm are subtoxic.
Before sacrifice, blood was withdrawn from orbital sinus of overnight fasting rats and serum was separated. After sacrifice of the animals, thyroid, liver and aorta were removed and homogenates (10%) were prepared in Tris-HCl buffer (10 mM, pH 7.4).

**Serum total cholesterol and triglyceride**

Total cholesterol was estimated according to the method of Chiamori and Henry. Triglyceride levels were estimated using glycerol phosphate oxidase-peroxidase (GPO-POD) based Enzokit supplied by Ranbaxy Diagnostic Ltd. (India) (Cat. No. D12603).

**Serum selenium levels**

Selenium levels were estimated by fluorimetric method. Serum samples were digested in modified Kjeldahl type flasks with reflux condenser attached to its neck to prevent any loss of selenium as volatile selenides.

**Selenium dependent GSH-Px activity**

Activity of GSH-Px was assayed by the coupled enzyme procedure with glutathione reductase using hydrogen peroxide (H₂O₂) as substrate. The assay was carried out in the post-mitochondrial fraction (PMF) of liver and aorta and the activity was expressed as μmoles of NADPH oxidized/min/mg protein.

**Low density lipoprotein (LDL) receptor level**

LDL receptor (LDL-R) levels quantitated in vivo. Human LDL was radioiodinated with Na[¹²⁵I] using the chloramine-T method. LDL-R level measurement was done by injecting (i.v.) 0.3 ml (Sp. activity 118 μCi/µg) of filter sterilized [¹²⁵I]-LDL to rat through penile vein. Counts per ml blood after 2 hr of i.v. injection was considered as counts at zero time or total initial counts in the blood. One ml blood was withdrawn from the orbital sinus of each rat after every two days and [¹²⁵I] counts were measured using a well type ¹²⁵I-gamma counter (Electronic Corp of India Ltd, India). This procedure was repeated every two days till the counts in any one group were reduced to 10% of the total initial counts. Per cent decrease in [¹²⁵I]-LDL counts at increasing time intervals was used as a measure of clearance rate of LDL from blood.

**T₃ and T₄ levels**

T₃ and T₄ levels were determined in serum by standard radioimmunoassay (RIA) kit obtained from Bhabha Atomic Research Centre, Mumbai, India (Cat No. RIAK-4A for T₃ and RIAK-5A for T₄).

**Total protein estimation**

Protein estimation in serum and in tissue homogenates was done by using the modified method of Lees and Paxman.

**Type-I iodothyronine 5'·deiodinase (5'-DI) activity**

5'·DI activity in tissue homogenates was determined by the modified method of Behne et al. The samples (1.0 mg protein) were incubated in a final volume of 0.5 ml of 100 mM Tris-HCl buffer (pH 7.4) containing 1 μM T₄ and 1 mM dithiothreitol at 37°C for 60 min. Chilled ethanol (1 ml) was added and T₃ production was determined in vitro in the ethanolic extracts by radioimmunoassay (RIA) kit. Heat denatured protein sample was used as background control.

**Statistical analysis**

Data are expressed as mean ± SEM. Difference between different groups were tested using Student’s t test for unpaired values.

**Results**

There was about sixteen fold increase in serum total cholesterol levels (Table 1) in animals fed on HFD as compared to control. Serum total cholesterol level in Se-supplemented group was significantly low in comparison with HFD fed group. Serum triglyceride levels (Table 1) also increased significantly with HFD feeding and were found to be near normal in animals fed with Se-supplemented HFD.

**Serum selenium levels**

Selenium was estimated in rat serum in all the three groups. A significant decrease in serum selenium levels was seen in animals fed on HFD when compared to control. Serum total cholesterol level in Se-supplemented group was significantly low in comparison with HFD fed group. Serum triglyceride levels (Table 1) also increased significantly with HFD feeding and were found to be near normal in animals fed with Se-supplemented HFD.

**Se-dependent GSH-Px activity**

In HFD group there was significant increase in the Se dependent-GSH-Px activity in the liver when compared to control (Table 1). In Se-supplemented HFD group, there was significant increase in serum Se levels when compared to both control and HFD group.

**Se-dependent GSH-Px activity**

In HFD group there was significant increase in the Se dependent-GSH-Px activity in the liver when compared to control (Table 1). Even more significant increase in the enzyme activity was observed in the liver of HFD+Se fed group. The enzyme activity in aorta showed similar trend as found in liver (Table 1), the changes being more significant.
Supplementation with Se along with the HFD had significant effect on increasing the deiodinase activity in liver in comparison to animals fed HFD alone (Table 2). Aortic deiodinase activity in HFD group also followed a similar trend as in liver. However, deiodinase activity in thyroid decreased significantly.

### Table 1 — Lipid profile, serum selenium levels and GSH-Px activity in rats after HFD and HFD+Se feeding [Values are mean±SEM]

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>HFD group</th>
<th>HFD+Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/dl; n=6)</td>
<td>55.5±7.2</td>
<td>919.5±37.9***</td>
<td>573.9±53.3***</td>
</tr>
<tr>
<td>Serum triglyceride (mg/dl; n=6)</td>
<td>68±7</td>
<td>153±10***</td>
<td>76±8***</td>
</tr>
<tr>
<td>Serum selenium (µg/l; n=10)</td>
<td>82.9±0.6</td>
<td>77.6±0.7***</td>
<td>107.9±1.2***</td>
</tr>
</tbody>
</table>

- Glutathione peroxidase (µmole of NADPH oxidised/min/mg protein; n=6)
  - Liver: 441±18 vs 549±29 vs 644±15
  - Aorta: 196±9 vs 279±19** vs 482±48***

- LDL receptor levels

  Rate of LDL clearance (Fig. 1) from blood was fastest in the control animals. Only 8.4% activity remained in blood after seven days. Inclusion of HFD in diet delayed LDL clearance as 18.7% activity was still present in blood after seven days. Supplementation of Se along with HFD only marginally increased this rate of clearance as 15.6% activity remained in blood after seven days.

- Serum T₃ levels

  Levels of serum T₃ decreased significantly in HFD group when compared to control (Table 2). In Se-supplemented HFD group however, T₃ levels were significantly higher than that of HFD group.

- Serum T₄ levels in serum

  As in the case of T₃, HFD feeding resulted in significant decrease in circulating T₄ levels when compared to control animals (Table 2). There was also a marked increase in the circulating T₄ levels in Se-supplemented HFD group when compared to HFD group.

- Type-I-5'-monodeiodinase activity

  The activity of 5'-deiodinase (Table 2) decreased significantly, in liver of HFD fed animals, in comparison to control. However, in aorta of the same group, there was no significant change in the activity of 5'-deiodinase. Deiodinase activity in thyroid of HFD group increased significantly in comparison to control.
in Se-supplemented HFD group (Table 2) when compared to that of HFD group.

Discussion

Hyperlipidemia is associated with hypothyroidism resulting from decreased clearance of both LDL and HDL by a defect in high affinity receptor mediated pathway of LDL catabolism\(^{16}\). T\(_3\) is known to increase LDL-R level which has long been proved to be protective against cholesterol induced atherosclerosis owing to its auto regulation phenomenon\(^{14,17}\).

In the present study we note significant decrease in the levels of T\(_3\) and T\(_4\) along with LDL-R levels in response to HFD feeding, T\(_4\) being a major secretory product of thyroid, the data indicate that hypercholesterolemia induces hypothyroidism, substantiating the earlier reported findings\(^3\). Further, levels of T\(_3\), T\(_4\) and LDL-R were found to be normal when Se was supplemented with HFD.

Decrease in the circulating T\(_3\) levels can be due to decrease in the activity of enzyme 5'-DI which is responsible for T\(_4\) to T\(_3\) monodeiodination. More than 85% of T\(_3\) production is reported to occur in extrathyroidal tissues such as liver, the HFD associated decrease in tissue 5'-DI activity support the above statement. Further the decreased T\(_3\) levels possibly led to decrease in LDL-R which are known to be regulated by T\(_3\) levels.

Activity of 5'-DI was significantly low in liver and aorta of HFD fed animals. Hypercholesterolemia and cardiovascular disorders have been shown to be associated with Se deficiency\(^{18,19}\) which is seen in the present study also as there is decreased serum selenium levels in HFD fed animals. Since Se has been reported to be an essential component of 5'-DI and is required for its enzymatic activity, Se deficiency associated with hypercholesterolemia might effect 5'-DI activity adversely. In our study, there was increased deiodinase activity in liver and aorta in Se-supplemented HFD group. This supports the reports of rapid decrease in 5'-DI activity in rat liver and kidney during Se depletion\(^{20,21}\).

However, levels of 5'-DI in thyroid were significantly higher in animals fed HFD. Thyroid unlike other peripheral tissues has been reported to maintain 5'-DI levels during drastic Se depletion\(^{21}\) suggesting thereby that thyroid has a higher priority in sequestering Se when its availability is very low\(^{22}\). It is not clear if the high level of glutathione peroxidase found in the thyroid provide a Se source for maintenance of 5'-DI activity\(^{23}\).

Se is a structural component of enzyme GSH-Px, which is known to subside the free radical stress. The increased GSH-Px activity found in the aorta and liver HFD fed animals could possibly be due to oxidative stress during HFD feeding. Increased GSH-Px activity in turn may result in Se depletion for other selenoproteins like 5'-DI and thus explains the decreased 5'-DI activity during HFD feeding. These findings also support the report\(^{18}\) that Se deficient state is induced by HFD feeding. GSH-Px levels increased significantly with Se+HFD feeding in our study. Since, Se equivalent to 1 ppm was supplemented with HFD whereas the adequate levels is 0.2 ppm Se in diet, the high Se supplementation probably results in higher level of GSH-Px activity in the Se supplemented group of animals in our study. The same explanation may hold good for the increased activity of 5'-DI in extrathyroidal tissues of Se-supplemented HFD group of animals.

Acknowledgement

Present study was financially supported by the Department of Atomic Energy, Govt. of India, Mumbai (India).

References

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