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THE EFFECT OF LONG-TERM SELENIUM AND VITAMIN E-ENRICHEO DIET ON THE CONTENT OF LIPID PEROXIDES AND CHOLESTEROL IN RATS

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Panczenko-Kresowska, B. and Ziemlański S.: The effect of long-term selenium and vitamin E-enriched diet on the content of lipid peroxides and cholesterol in rats. Acta physiol. pol., 1987, 38(4): 346—352. The effect of long-term diets enriched with natural antioxidants was studied on Wistar rats with average initial body weight 150 g. After enrichment of the diet with selenium (0.1 ppm of sodium selenite per 100 g of diet), with vitamin E (6 mg of alpha-tocopherol per 100 g of diet) and selenium and vitamin E together the following results were obtained: diets enriched with selenium or vitamin E given for 12 months reduced the production of lipid peroxides in the liver and serum of the rats. On the other hand, addition of both antioxidants to the diet had no effect on lipid peroxide levels in the animals. Diet enrichment for 12 and 18 months with selenium or vitamin E had no effect on the levels of total cholesterol and HDL cholesterol. The obtained results suggest that selenium and alpha-tocopherol exert an inhibitory action on the processes of ageing in the experimental animal model.

Key words: vitamin E; selenium; lipid peroxide; cholesterol; ageing

The importance of selenium and vitamin E for the normal functioning of cell enzymes and biological membranes has been recently the subject of interest. Selenium being an integral part of glutathione peroxidase is the second line of cell defense against uncontrolled oxidation of lipids, mainly multiradical. The defensive role of alpha-tocopherol (vitamin E) in the processes of lipid peroxide formation is based, among others, on an inhibition of the development of the peroxides of polyunsaturated fatty acids in the phospholipids of biological membranes [Opieńska-Blauth, J. et. al., 1980; Ziemlański and Wartanowicz 1982].

It has been demonstrated in many experimental studies that diet enrichment with certain natural or synthetic antioxidants increases the

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mean life span of mice and rats [Khon, R. R., 1971; Oerin, S. and Vochitu, E., 1965; Porta, E. A. et al., 1980; Shroeder, H. A., 1971]. These observations as well as the theory of Herman (1972) on a harmful effect of free radicals in the processes of ageing of the organism suggest an important role of the natural antioxidants in the protection of the organism against the degeneration-inducing activities of lipid peroxides. This hypothesis has gained support by the observations indicating that the tissue contents of vitamin C, selenium and peroxide dysmutase decrease with progressing ageing of the organism [Ziemlański and Wartanowicz, 1982].

The purpose of this study was to investigate the effect of the addition of biological antioxidants such as selenium and vitamin E to diets on the levels of lipid peroxides and cholesterol in rats.

MATERIAL AND METHODS

Investigations were carried out on 120 male Wistar rats obtained from the breeding farm of the National Institute of Food and Nutrition. Their initial body weight was 150 g. The rats received semisynthetic diets enriched with selenium or vitamin E or with both these antioxidants jointly during 12 and 18 months. After 12 and 18 months of the experiment the rats were killed by decapitation and their blood and livers were taken for biochemical investigations. All the animals in the experimental group had free access to food and water. They were kept in cages of $40 \times 35 \times 25$ cm in dimensions with 5 animals in each cage. The light-darkness periods were 12—12 hours, the ambient temperature was 21—23°C and air humidity was 70%.

Experimental diets. The semisynthetic experimental diet consisted of Murigran food with added powdered hen eggs, casein and sunflower seed oil in the following proportions per 100 g of the diet: Murigran — 88 g, egg powder — 6 g, casein — 2 g, sunflower oil — 4 g. The diet contained: protein 22% ; fat 9%; 2% of energy was derived from essential unsaturated fatty acids (EFA). This diet was enriched with selenium by adding sodium selenite 0.1 ppm or with vitamin E (alpha-tocopherol) 6 mg per 100 g of the diet, or both these substances were added in the above doses. The control diet was the semisynthetic food without added selenium or vitamin E.

Murigran was obtained from the Bacutil Animal Food Industry Corporation. It was prepared according to the formula No 1/113980.

During the whole experiment the body weight of the rats was measured once in a week.

At the chosen time intervals the animals were decapitated after starving them for 12 hours, and the blood and liver were taken for investigations.

In the serum and liver the levels of lipid peroxides measured as malonyldialdehyde were determined by the method of Satoh (1978). The blood samples were deproteinized with 20% trichloracetic acid, the liver was homogenized in 0.9% saline in a 1:10 proportion, and deproteinized with 10% trichloracetic acid.

Concentrations of total cholesterol and HDL cholesterol fraction were determined by the method of Błaszczyszyn (1970). HDL fraction was obtained by precipitation of the lighter serum fractions with sodium phosphotungstenate [Burstein et al., 1970].

The statistical significance of the obtained differences was calculated using the Student test for unpaired samples. The results were expressed as means ± SD.
RESULTS

Table 1 shows the results of determinations of the end body weight of the rats in this experiment.

Table 1. Mean body weights of rats receiving experimental diets enriched with selenium or vitamin E (means ± SD)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Final body weight (g)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>12 months</td>
<td>n</td>
<td>18 months</td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td>432±23</td>
<td>14</td>
<td>364±57**</td>
</tr>
<tr>
<td>with added:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>selenium</td>
<td>14</td>
<td>453±53</td>
<td>14</td>
<td>431±47</td>
</tr>
<tr>
<td>vitamin E</td>
<td>15</td>
<td>447±42</td>
<td>14</td>
<td>434±47</td>
</tr>
<tr>
<td>selenium + vitamin E</td>
<td>15</td>
<td>462±37</td>
<td>15</td>
<td>420±49</td>
</tr>
</tbody>
</table>

** p < 0.01 in relation to the value at 12 months.

No effect of selenium and vitamin E on the final body weight of the experimental rats was found. A tendency was noticed for a gradual loss of body mass with progressing age of the rats (Tab. 1).

The levels of lipid peroxides in the serum and liver of the experimental rats are presented in Table 2.

Table 2. Levels of lipid peroxides in the serum and liver in rats kept on experimental diets enriched with selenium or vitamin E (means ± SD)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Lipid peroxidesb</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>serum nm/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>12 months</td>
<td>n</td>
<td>18 months</td>
<td>n</td>
<td>12 months</td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td>3.50±0.20</td>
<td>14</td>
<td>3.49±0.39</td>
<td>178.8±39.2</td>
<td>163.7±23.6</td>
</tr>
<tr>
<td>with added:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>selenium</td>
<td>14</td>
<td>2.95±0.41*</td>
<td>14</td>
<td>2.99±0.36</td>
<td>121.0±18.1</td>
<td>133.9±29.6*</td>
</tr>
<tr>
<td>vitamin E</td>
<td>15</td>
<td>2.75±0.63*</td>
<td>13</td>
<td>2.46±0.35**</td>
<td>106.2±34.7*</td>
<td>142.0±27.6</td>
</tr>
<tr>
<td>selenium + vitamin E</td>
<td>15</td>
<td>3.61±0.26</td>
<td>12</td>
<td>3.26±0.37</td>
<td>137.9±31.9</td>
<td>152.4±27.0</td>
</tr>
</tbody>
</table>

b expressed as malonyldialdehyde
* p < 0.05 in relation to control group
** p < 0.01 in relation to control group

Addition of selenium 0,1 ppm or vitamin E 6 mg to the diet decreased the serum level of lipid peroxides after 12 months of the experiment. It was also demonstrated that with longer duration of the experiment the effect of vitamin E as an antioxidant of liver peroxides increased. The
concentration of lipid peroxides in the serum of the animals kept during 18 months on the diet enriched with vitamin E decreased further, and this decrease was statistically significant in relation to the value obtained after 12 months of the experiment. Addition of both selenium and vitamin E to the diet had no effect on the level of serum lipid peroxides.

Long-term feeding with diets enriched with selenium or vitamin E caused changes in the level of lipid peroxides in the liver (Tab. 2). It was found that addition of the biological antioxidants to the diets inhibited significantly the accumulation of peroxides in the liver of the experimental animals kept during 12 months on diets with added selenium or vitamin E. After 18 months of the experiment the inhibitory effect of selenium and vitamin E on the accumulation of lipid peroxides in the liver was not statistically significant in both experimental groups, however, the concentration of peroxides was lower than in the control group. No decrease of the concentration of lipid peroxides was observed after 12 and 18 months in the livers of rats fed on the diet containing both selenium and vitamin E.

Table 3. Serum levels of total cholesterol and HDL-cholesterol in rats receiving experimental diets enriched with selenium or vitamin E (means ± SD)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Total cholesterol (mg/dl)</th>
<th>HDL-cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n 12 months</td>
<td>n 18 months 12 months</td>
</tr>
<tr>
<td>Control</td>
<td>15 103.0±17.8</td>
<td>14 134.2±12.5*</td>
</tr>
<tr>
<td>with added:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>selenium</td>
<td>14 90.6±20.0</td>
<td>14 93.5±14.0**</td>
</tr>
<tr>
<td>vitamin E</td>
<td>15 121.2±20.9</td>
<td>13 122.8±21.8</td>
</tr>
<tr>
<td>selenium + vitamin E</td>
<td>15 116.0±11.6</td>
<td>12 109.6±15.1</td>
</tr>
</tbody>
</table>

* p < 0.01 in relation to rats killed after 12 months
** p < 0.01 in relation to control group

The addition of selenium or vitamin E to the diet had no significant effect on the serum levels of cholesterol in the studied animals (Tab. 3). The levels of total cholesterol and HDL cholesterol were similar to those found in rats aged 12 or 18 months. On the other hand, a statistically significant rise was noticed of the total cholesterol level in the serum of the control rats on the 18th day of the experiment as compared with the value obtained after 12 months of the experiment.
DISCUSSION

Changes occurring in the concentrations of certain lipid fractions in the process of ageing of the organism have been studied for a long time. A rise was observed in the level of total cholesterol with a fall of HDL cholesterol in old humans and animals as compared to young ones [Jamamoto and Yamamura, 1971; Keys et al., 1950; Kritchewsky, 1979]. In ageing rats a decrease was demonstrated in the concentration of phospholipids in the liver [Hawcroft and Martin, 1974]. Many authors have reported increased levels of lipid peroxides in the serum of old subjects [Suematsu et al., 1977; Wartonowicz et al., 1984; Yoshikawa and Hirai, 1967], and ageing animals [Grinna and Barber, 1973; Takeuchi et al., 1976, Takeuchi et al., 1978]. In many tissues, especially in the brain of ageing animals accumulation was observed of the "senility pigment" (lipofuscin developing as a result of polymerization of proteins with lipid peroxides) [Yoshikawa and Hirai, 1967; Taubold et al., 1975].

Tappel et al. (1974) and Reddy et al. (1973) demonstrated that administration of alpha-tocopherol prevented the development of lipofuscin in the tissues of the experimental animals. At the same time it was demonstrated in animal experiments that addition of natural or synthetic antioxidants to diet reduced the concentration of certain lipids in the serum and tissues of the studied animals.

Chen et al. (1972) noted that vitamin E decreased the serum cholesterol level in rats. The studies of Hafeman (1977) and Dillard (1978) demonstrated that animals kept on diets with low selenium and vitamin E content produced many times more pentane as compared to the animals receiving diets enriched with selenium or vitamin E.

Harman (1972) showed that certain antioxidants prolonged the life of mice by 7—26%/ depending on the compound administered to them.

This survey of the pertinent literature and the present results suggest that diet enrichment with natural antioxidants such as vitamin C [Wartonowicz et al., 1984], vitamin E or selenium exerts an inhibitory effect on the production of lipid peroxides in the organism of humans and animals. Of the two antioxidants used in this study vitamin E seems to be a more powerful antioxidant than selenium. This was particularly evident in the serum of the rats examined. Alpha-tocopherol reduced the concentrations of both free radicals and lipid peroxides. As it was shown by Ziemlański et al. (1982) each particle of vitamin E is capable of binding 100 or more molecules of peroxide radicals. The action of vitamin E in the organism is aided by other antioxidants, such as selenium, peroxide dysmutase or sulphur-containing amino acids. In our experiments on rats we failed to demonstrated a decrease in the concentration of lipid peroxides in rats fed on the diet enriched with both selenium.
and vitamin E. This is difficult to explain since we expected a synergistic action of both these antioxidants. In our earlier report [Wartanowicz et al., 1984] in which vitamin C effect was described in an experiment on elderly subjects given his vitamin during one year, the reduction of serum lipid peroxide concentration was largest when vitamin C was taken together with vitamin E.

Our present results showed also that the serum levels of total cholesterol and HDL cholesterol were unchanged after addition of antioxidants to the diet.

Of particular interest is, however, the observation that in the group of rats receiving diets with selenium and vitamin E the concentration of serum cholesterol was not increasing with age. A continuous rise of cholesterol was demonstrated, on the other hand, in aging control rats.

In conclusion, diets enriched with selenium or vitamin E cause an inhibition of the lipid peroxide formation in the rat serum and liver. Long-term administration of moderate doses of selenium or vitamin E delayed the rise of serum total cholesterol concentration connected with the process of animal ageing. Both these findings indicate that selenium and alpha-tocopherol exert an inhibitory effect on the process of ageing of the organism in model experiments on animals.

REFERENCES


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