**Effect of vitamin E supplementation on α-tocopherol status and tissue antioxidants in American minks (Neovison vison)**

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Abstract: The α-tocopherol (TCP) state as well as enzymatic (superoxide dismutase [SOD] and catalase [CAT]) and nonenzymatic (reduced glutathione [GSH] and retinol) antioxidants in tissues of American minks (Neovison vison) given various levels of dietary vitamin E were examined. Three groups of farm-bred minks received different amounts of DL-α-tocopheryl acetate (10 mg/kg diet, control; 30 and 110 mg/kg diet, treatments denoted as E30 and E110, respectively) for 14 days in November. The TCP content was raised substantially in the kidneys and reduced in the livers of minks in the E110 group. The action of the 2 vitamin E doses on antioxidants was similar. The treatments resulted in lower hepatic and kidney retinol levels and hepatic and skeletal muscle SOD, as well as hepatic CAT activities, higher heart SOD activity, and higher kidney GSH content in comparison to the control group. The present results show that dietary vitamin E intake over 2 weeks considerably changed the TCP status of the liver and kidneys and had tissue-specific action on measured antioxidants in minks. With the exception of the heart SOD activity, the additional intake of vitamin E reduced antioxidant enzyme activity and augmented the GSH level, indicating an enhancement in the nonenzymatic part of the antioxidant network.

Key words: Vitamin E, retinol, catalase, glutathione, superoxide dismutase, mink

1. Introduction

Being a naturally occurring nutrient, vitamin E performs multiple functions in the mammalian organism, playing a significant role in metabolic processes. It is the major lipid-soluble component in cell membranes and circulating lipoproteins. Its antioxidant action can be viewed as a regulatory compound that helps to maintain free radical reactions at a certain steady level (1,2). Vitamin E has also been shown to influence redox-regulated gene expression (3). Among all forms of vitamin E, α-tocopherol is specifically retained in the body and exerts the highest biological activity (4).

The mammalian organism does not synthesize vitamin E; therefore, it is an essential component of the diet, the demand for which depends on a number of factors, such as season, physiological state, nutritional value, and composition of diet. The addition of synthetic analogs of vitamin E to farm-raised animals’ diets is a common practice to avoid hypo- and avitaminosis, to enhance growth and reproduction, and to prevent diseases such as muscular dystrophy, steatitis, and sterility (5–7). The capacity to accumulate this nutrient is rather limited. Since components of the antioxidant system (AOS) are mutually compensatory, an artificial rise of one antioxidant in an organism may affect the content of others. Even if information about the action and metabolism of vitamin E in various animal species is plentiful (2,4,8), its interactions with other antioxidants in carnivorous fur animals have yet to be elucidated. Previously reported research on vitamin E addition in minks generally described the vitamin E status under dietary oxidative stress (9,10). The aim of this experiment was to determine the influence of various levels of dietary vitamin E on TCP status and tissues’ enzymatic (SOD and CAT) and nonenzymatic (GSH and retinol) antioxidants in farmed American minks under normal conditions.

2. Materials and methods

2.1. Animals and diets

Six-month-old farm-raised standard dark-brown American minks (Neovison vison) were used in this study. The animals were kept in pairs, 1 male and 1 female per cage, in accordance with normal farming practices, and fed a paste-like diet and water ad libitum. Minks with approximately the same weight were randomly divided into 3 groups and fed diets supplemented with 10 (control, n = 6), 30 (E30, n = 10), and 110 (E110, n = 10) mg of vitamin E...
(DL-α-tocopheryl acetate, Cuxavit E 50, Kaesler Nutrition GmbH, Cuxhaven, Germany) per kilogram of feed for 14 days in November. The doses of the experimental groups (E30 and E110) were respectively 3 and 11 times the amount added during the autumn on the fur farms where the experimental animals were raised. The average weight of the mink males and females was 1984 ± 195 g and 1264 ± 123 g, respectively; the sex ratio in each group was 1:1. The minks used for the study were all in good health and showed no signs of illness throughout the experiment. The composition of the basal diet (BD) is shown in Table 1. All the experimental procedures were performed according to EU guidelines on the use of animals for biochemical research (86/609/EU).

2.2. Sampling procedures
At the end of the experiment samples of blood were obtained from the tail tip in the morning after a night of fasting. Serum was separated by centrifugation at 1500 × g for 10 min. Samples of the liver, kidneys, heart, skeletal muscle, lungs, and spleen were gathered during the standard fur production process. The samples of serum and tissues were stored until analysis at –25 °C. All analyses were performed within a month after the collection of the samples.

2.3. Biochemical analysis
The research was conducted using the equipment of the Core Facility of the Karelian Research Centre of the Russian Academy of Sciences. The concentrations of TCP and retinol in blood serum and tissues were determined by HPLC. The samples of tissues were homogenized in 0.25 M sucrose solution (pH 7.4), and serum was mixed with ethanol containing an antioxidant (butylated hydroxytoluene) to precipitate proteins. After that, n-hexane was added. The mixture was vortexed for 5 min for the extraction of vitamins and centrifuged at 3000 × g for 10 min. The hexane layer was injected onto the HPLC system. Chromatographic separation was carried out by microcolumn chromatography with a UV detector with n-hexane and isopropanol as an eluent (98.5:1.5). The eluate was monitored at 292 nm for TCP and 324 nm for retinol, and the vitamins were identified by retention time compared with pure standards (MP Biodicals). Superoxide dismutase and CAT activities and GSH content in tissues were determined according to Sergina et al. (11). The level of GSH was determined using only the liver, kidneys, and heart.

2.4. Statistical analysis
Preliminary tests revealed no differences between the sexes; hence, data for males and females were pooled in all subsequent analyses. Results of the study were transformed into SI units and processed statistically as mean ± standard error of the mean. Statistical analysis was conducted using the Wilcoxon–Mann–Whitney test. Significance of differences between samples was considered for P < 0.05. The statistical analyses were performed using SigmaStat 2.03 (Jandel Scientific, Erkrath, Germany).

3. Results
As shown in Table 2, the serum TCP concentration caused an upward tendency in the E110 group in comparison to the control group. There was a significant difference in the serum TCP content between the E30 and E110 groups (P < 0.05).

In control minks, a relatively low TCP level was detected in the cardiac and skeletal muscles among all investigated tissues. There was a significant (P < 0.05) reduction in the liver and a rise in the kidney TCP contents in the E110 group compared with the control values. The same tendency was seen for the E30 group. The level of TCP

Table 1. Composition of the basal diet.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Content [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat meal</td>
<td>33</td>
</tr>
<tr>
<td>Bone and meat meal</td>
<td>9</td>
</tr>
<tr>
<td>Fish meal</td>
<td>12</td>
</tr>
<tr>
<td>Extruded corn</td>
<td>38</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>2</td>
</tr>
<tr>
<td>Dried yeast</td>
<td>6</td>
</tr>
<tr>
<td>Vitamin-mineral premix*</td>
<td>0.2</td>
</tr>
<tr>
<td>Metabolic Energy (ME) [MJ/kg]</td>
<td>5.23</td>
</tr>
<tr>
<td>% ME from: Protein</td>
<td>46</td>
</tr>
<tr>
<td>Fat</td>
<td>38</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>16</td>
</tr>
</tbody>
</table>

*Provided per kilogram of diet: vitamin A, 1250 IU; vitamin B1, 0.5 mg; vitamin B2, 0.5 mg; vitamin B6, 2.5 mg; Fe, 50 mg; Mn, 2.5 mg; Cu, 2 mg; Zn, 0.3 mg; I, 0.1 mg; Se, 0.03 mg.

Table 2. The concentrations of α-tocopherol and retinol in blood serum of minks, µmol/L (means ± standard error of the mean).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Control</th>
<th>E30'</th>
<th>E110'</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Tocopherol</td>
<td>16.89 ± 3.57ab</td>
<td>9.64 ± 1.18b</td>
<td>37.05 ± 13.79b</td>
<td></td>
</tr>
<tr>
<td>Retinol</td>
<td>1.36 ± 0.16</td>
<td>1.03 ± 0.13</td>
<td>1.26 ± 0.14</td>
<td></td>
</tr>
</tbody>
</table>

*E30, the group of minks administered 30 mg vitamin E per kg basal diet; #E110, the group of minks administered 110 mg vitamin E per kg basal diet; a, b: values in the same line with different letters differ significantly at P < 0.05.
in the heart, lungs, and spleen did not differ substantially among the experimental groups. Skeletal muscle vitamin content was considerably greater in minks fed the E110 diet than in minks fed the E30 diet (P < 0.05) and the control group (Figure 1).

As shown in Table 3, the retinol content was the highest in the kidneys among all tissues, and it was undetectable in the skeletal muscle and lungs. The retinol level diminished significantly (P < 0.05) in the liver and kidneys with an increasing amount of dietary vitamin E supplementation.

The increase of vitamin E content in the BD caused significant reduction in the activity of SOD in the livers and skeletal muscles of minks in the E30 and E110 groups (Figure 2a, P < 0.05). The SOD activity was lower (P < 0.05) in the spleens of minks fed the E30 diet than those of the control minks. In the heart, where the activity of SOD was relatively low in the control minks, it was augmented in both supplemented groups (P < 0.05). SOD status in the kidneys and lungs was not affected by dietary vitamin E. The CAT activity was decreased (P < 0.05) in the livers in the E30 and E110 groups in a dose-dependent manner, and in the hearts of minks fed the E30 diet (Figure 2b). There were also differences in the enzyme activity between the E30 and E110 groups in the heart and spleen (P < 0.05). No substantial changes in the CAT activity were seen in the kidneys, lungs, and skeletal muscles of the experimental groups.

The level of GSH was the highest in the heart among examined tissues of the control minks (Figure 3). After 2 weeks of the E30 diet, GSH content was elevated in the kidneys when compared with the control group; in the E110 group, augmentation was observed in both the kidneys and heart when compared with the control group (P < 0.05).

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**Table 3.** The content of retinol in tissues of minks, µg/g wet tissue (means ± standard error of the mean).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Groups</th>
<th>E30</th>
<th>E110</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>Control</td>
<td>16.11 ± 1.68a</td>
<td>11.19 ± 0.98b</td>
</tr>
<tr>
<td>Kidney</td>
<td>E30</td>
<td>199.16 ± 8.66a</td>
<td>160.25 ± 8.74b</td>
</tr>
<tr>
<td>Heart</td>
<td>E110</td>
<td>0.68 ± 0.12</td>
<td>0.55 ± 0.08</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td>1.06 ± 0.43</td>
<td>2.45 ± 1.32</td>
</tr>
</tbody>
</table>

*E30, the group of minks administered 30 mg vitamin E/kg basal diet; #E110, the group of minks administered 110 mg vitamin E/kg basal diet; a, b: values in the same line with different letters differ significantly at P < 0.05.
**Figure 2.** The superoxide dismutase (a) and catalase (b) activities in tissues of minks (means ± standard error of the mean).

**Figure 3.** The level of glutathione in tissues of minks (means ± standard error of the mean).
4. Discussion
In our study, the level of TCP in serum in minks fed diets with 30 or 110 mg of vitamin E per kilogram of feed for 2 weeks did not differ significantly from the control, except for an upward tendency in the group that received the highest dose. Työppönen et al. (12) reported that in minks supplemented with 150 mg of α-tocopheryl acetate per kilogram of BD for 5 months, plasma TCP concentration increased 2.5 times. Our results showed there was no direct correlation between daily intake of vitamin E and its concentration in blood, since the newly absorbed vitamin partially replaces the TCP in circulating lipoproteins (4). Another reason for the insignificant change of the TCP level in serum might be the short duration of vitamin E exposure.

The intake of 110 mg of DL-α-tocopheryl acetate per 1 kg BD changed the TCP contents in the livers and kidneys of the minks. The liver regulates vitamin E metabolism and its status in the body (4). This organ, like adipose tissue, is able to accumulate vitamin E (8,12). We did not observe TCP augmentation in the livers of the minks in either of the experimental groups. In this study, its level was lower than in the control animals. A similar result was reported by Leonard et al. (13) for the livers of rats. It was established that the surplus of vitamin E causes an upregulation of TCP metabolism and/or activation of pathways to dispose of TCP (13,14). Perhaps this mechanism was the reason for a significant decrease in TCP content in the E110 group. In agreement with this assumption, we observed a considerable rise in renal TCP level in minks fed the E110 diet. A similar result was reported by Lass et al. (15) for mice. In addition to biliary excretion, vitamin E metabolites are eliminated by the kidneys. Furthermore, vitamin E metabolism takes place within the kidneys (14).

Muscle tissue is highly susceptible to reactive oxygen species (ROS) and sensitive to dietary vitamin E status (6,16). Our results showed that cardiac TCP level did not differ significantly between groups of minks receiving different doses of DL-α-tocopheryl acetate, which was probably due to the relatively short feeding period. Rojas et al. (17) reported that the cardiac TCP contents of guinea pigs more than doubled after vitamin E administration (150 mg/kg diet) over the course of 5 weeks. While the level of TCP in the skeletal muscle was increased moderately in minks fed the E110 diet, it was not a statistically significant increase.

Although vitamin E is the most important lipophilic antioxidant for alveolar surfactants (18) and an essential antioxidant and modulator of the immune system (19), its addition to the BD of minks did not influence TCP level in the lungs and spleen. After the addition of vitamin E to the diet, the level of TCP in tissues changed relatively slowly because of the lipid solubility of this nutrient and the rate of its metabolism (17). In minks, the physiological demand for vitamin E has been estimated to be 18–25 mg/kg of feed (12,20). The short-term supplementation with 110 mg of vitamin E per 1 kg BD used in our study significantly changed the TCP status in the liver and kidneys, and the same tendency was detected for the 30 mg/kg dose. It is to be noted that minks have a great capacity for fat storage in autumn (35%–38%) (21), and part of the vitamin E supplied to the diet could be taken up by adipose tissue for protection against peroxidative damage.

The antioxidant function of vitamin A in cells is associated with its ability to scavenge free radicals (22). In the present research, augmentation of the amount of vitamin E in the BD of minks caused no substantial changes in the blood retinol concentration, but its level was affected negatively in both the liver and the kidneys, which regulate vitamin A homeostasis. It is likely that a rise in dietary vitamin E dosage initiated competition between these two fat-soluble nutrients during intestinal absorption. Reboul and Borel (23) found that vitamin E was able to downregulate the expression of proteins involved in the uptake and basolateral secretion of β-carotene in the intestine. Moreover, vitamin E reduces the rate of hydrolysis of retinyl esters in the liver (24).

An increase in the dietary vitamin E content affected the activity of antioxidant enzymes in the liver, heart, skeletal muscle, and spleen. We found diminished hepatic activity of antioxidant enzymes in minks fed E30 and E110 diets. A suppressing effect of vitamin E supplementation on hepatic SOD activity has been demonstrated in rats and rabbits (25,26). It can be assumed that decline in antioxidant enzyme activity is due to changes in superoxide and peroxide production by cytochrome P450 during vitamin E metabolism (4,22). Superoxide dismutase and CAT activities in the kidneys were not influenced by additional vitamin E, but there was a simultaneous rise in the level of GSH, which is involved in the regeneration of oxidized vitamin E (17).

In both experimental groups, a considerable rise in SOD activity in the heart was observed. Since SOD synthesis is induced by production of superoxide (27), one can assume that this process was more active in the cardiomyocytes of the experimental animals. In contrast, it was shown that dietary vitamin E dose-dependently reduced the production and/or level of superoxide in the hearts of female rats (28). It can be assumed that there are species-specific differences in the reaction of antioxidants in the heart to vitamin E supplementation in rodents and carnivores. The reduced CAT activity in the hearts of minks fed the E30 diet can probably be explained by its depletion because of enhanced hydrogen peroxide generation. On the other hand, glutathione peroxidase has been shown to play a considerable role as an H₂O₂ scavenger in the heart,
since its activity is much higher than that of CAT (29). The rise of the cardiac GSH level in the E110 group together with the augmentation of SOD activity can be interpreted as activation of the antioxidant defense system. Published data (16,17) on the influence of vitamin E on the AOS in the heart are contradictory. For example, a stimulating effect of TCP on CAT and SOD activities in the hearts of rats of different ages was demonstrated by Asha Devi et al. (16), but Rojas et al. (17) reported that there was no change in individual endogenous antioxidants in the hearts of guinea pigs administered additional vitamin E. According to the same authors, vitamin E uptake exceeding the minimum daily demand by a factor of 6 enhanced total antioxidant capacity and ameliorated the redox status of cardiomyocytes. It appears that the effect of vitamin E is related to both dosage and species-dependent features.

Superoxide dismutase activity was decreased in both supplemented groups in comparison to the control group in the skeletal muscle as well as in the liver. We also found a significant decrease in SOD activity in the spleens of minks fed the E30 diet compared with the control group. The inhibition of SOD activity by vitamin E may be due to the ability of this nutrient to regulate the production and level of superoxide and the related ROS in the mitochondria by maintaining mitochondrial integrity and stability (30), as has been shown for rodent tissues (28).

The response of the antioxidants to dietary vitamin E supplementation in minks was tissue-specific. Since the liver is the main regulator of vitamin E homeostasis in the body, the addition of this nutrient to the diet was found to significantly change the investigated parameters in this organ in minks. In most cases, vitamin E supplementation lowered the activities of antioxidant enzymes in tissues, as far as the exclusion of myocardial SOD activity. Cardiac tissue is largely postmitotic and exhibits a highly aerobic metabolism due to the abundance of large mitochondria, whereas in the skeletal muscle as well as in the liver anaerobic energy systems prevail (31). In addition, the metabolic rate and energy demands of mustelids are elevated because of a high surface-to-volume ratio (21). Thus, the observed increase in SOD activity was probably connected with the metabolic features of cardiac cells, coupled with the fact that cardiomyocytes have a slow turnover of antioxidant enzymes (16). As stated by Nakamura and Omaye (3), where exogenous antioxidants are imbalanced or limited to reducing ROS, TCP may induce endogenous antioxidant enzymes. At the same time, the antioxidant parameters of the lungs were not affected by additional vitamin E. It was found that adequate control of oxidative balance is important for the maintenance of normal pulmonary cellular function (32); a longer exposure period is probably required to affect the antioxidants in the lung.

In the present study, differences between the medium (30 mg/kg BD) and high (110 mg/kg BD) doses of vitamin E were observed in relation to TCP level in the blood serum and skeletal muscle; the activity of CAT in the liver, heart, and spleen; and the level of GSH in the heart. It is to be noted that the modulating effect of dietary vitamin E on cardiac CAT and splenic SOD activities was detected only at the medium dose. It is known that the effect of vitamin E may depend on microenvironments, such as the ratio between the concentrations of vitamin E and other antioxidants. Along with its antioxidant action, TCP may play a role in modulating the expression of antioxidant defense enzymes as a gene regulator, and this effect may be controlled through multiple signaling pathways (3).

In summary, the present investigation showed that dietary supplementation of vitamin E over the course of 2 weeks affected TCP and the retinol status of the liver and kidneys, and had tissue-specific effects on the measured antioxidative parameters in minks. Except for the cardiac SOD activity, additional intake of vitamin E reduced the activities of antioxidant enzymes and augmented the level of GSH, indicating an enhancement in the nonenzymatic part of the antioxidant network. These results provide important information regarding the influence of vitamin E on the activities of antioxidant enzymes and the status of other antioxidants in minks.

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References