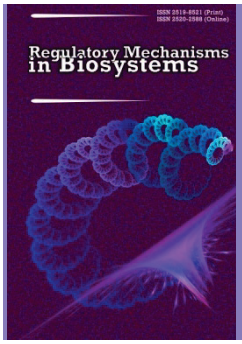

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The effect of vitamin E on the lipid environment of rat hepatocyte membranes

S. B. Silonov*, **, E. O. Kryvenko*, N. B. Silonova***, T. M. Shevchenko****

*Shupyk National Healthcare University of Ukraine, Kyiv, Ukraine
**Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine, Kyiv, Ukraine
***Ukrainian Scientific-Research and Training Centre for Standardization, Certification and Quality Problems, Kyiv, Ukraine
****Oles Honchar Dnipro National University, Dnipro, Ukraine

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Shupyk National Healthcare University of Ukraine, Dorohozhytska st., 9., Kyiv, 04112, Ukraine. Tel.; +38-097-306-88-96. E-mail: ssb@ukr.net

Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine, Leontovicha st., 9, Kyiv, 01030, Ukraine. Tel.: +38-097-232-54-95. E-mail: ssb@ua.fm

Ukrainian Scientific-Research and Training Centre for Standardization, Certification and Quality Problems, Svyatoshynska st., 2, Kyiv, 03115, Ukraine. Tel.: +38-067-768-24-19. E-mail: silonova@ukr.net

Oles Honchar Dnipro National University, Gagarin av., 72, Dnipro, 49010, Ukraine. Tel.: +38-067-566-05-42. E-mail:dnushevchenkot@gmail.com

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Tocopherol is one of the known beneficial natural antioxidants ensuring the optimal level of functioning of mammalian organisms. Numerous *in vitro* and *in vivo* experiments have shown that the biological role of vitamin E is to prevent the development of pathologies caused by oxidative stress. In particular, the role of enzymatic factors of lipid peroxidation and related inflammation as a result of eicosanoid synthesis was clearly shown. We studied changes in the structural and functional state of hepatocyte membranes in the classical model of E-hypovitaminosis caused by long-term (70 days) insufficient intake of vitamin E in the diet of rats. The test components were determined spectrophotometrically after appropriate chromatographic procedures. The amount of total and individual leukotrienes was determined by ELISA. Prolonged tocopherol deficiency in rats caused a 49.4% decrease in tocopherol, more than 27.0% – in cholesterol. Of the 8 individual phospholipids studied, 6 showed significant changes: a decrease in cardiolipin and phosphatidylserine, and an increase in phosphatidylethanolamine by 3.24 times, an increse in lysophosphatidylcholine by 86.9%, in phosphatidylcholine by 52.8%, and in sphingomyelin by 30.6%, relative to control. There were changes in the levels of unsaturated fatty acids playing a significant role in the development of functional disorders in cells and affecting the metabolism of ecosanoids derived from arachidonic acid by the 5-lipoxygenase oxidation pathway. Changes in the levels of total and individual cysteinyl leukotrienes in the state of E-hypovitaminosis were revealed. Restoration of vitamin E intake returns most of the studied indicators such as tocopherol, cholesterol, polyunsaturated fatty acids to the control levels and activates the processes of sequential conversion of leukotrienes in the body of rats. The obtained results indicate the potentiating effect of vitamin E on metabolic processes in the body as a whole and in hepatocytes and eicosanoid metabolism. The degree of tocopherol intake allows one to influence the course of inflammatory processes associated with eicosanoids, not only through the impact on precursors, but also on the utilization of metabolites, including leukotrienes.

Keywords: cholesterol; hepatocyte membrane phospholipids; arachidonic acid; total cysteinyl leukotrienes; individual cysteinyl leukotrienes.

Introduction

It has been 100 years since Evans and Bishop discovered vitamin E and described it as a nutritional factor necessary for the normal reproduction of rats. It took more than 10 years to create the chemical and later structural formula of α-tocopherol. Olcott later described the antioxidant properties of tocopherol and continued to study its biological role. During this long period, a lot of information has been accumulated about the role of vitamin E in *in vivo* and *in vitro* systems.

Over the last few decades, researchers' interest in the biological importance of vitamin E has also been growing. Vitamin E is known to combine 8 natural vitamins (4 tocopherols and 4 tocotrienols), which are derivatives of tocol alcohol. They are characterized by different biological activity both at the level of the organism as a whole and at the level of individual cells (Packer, 1994). In nature, there are only free forms of tocopherol, its esters are obtained synthetically, they show much lower activity of vitamin E, the rate of their hydrolysis is a factor that determines the potential of vitamin E in the body and is largely controlled by extracellular Ca^{2+} (Brigelius et al., 2002). It is known that α-tocopherol is the most common component of vitamin E in nature and in mammals (Chin & Ima-Nirwana, 2014). Vitamin E is not synthesized in humans and animals (Guo et al., 2006), while plant organisms, due to their ability to photosynthesis, are able to synthesize tocopherol (Birringer et al., 2018). According to the results of an analytical study, the maximum content of natural tocopherol was found in oilseeds and oil fraction of nuts (Shahidi & Camargo, 2016; Vanik et al., 2017). The main sources of tocopherols are almond oil and other nut oils, olives, sunflowers, rapeseed, corn, flaxseed and soybean oils. At the same time, tocotrienols can be found in the oils of palm and rice bran, wheat germ, barley, oats, hazelnuts, corn (Shahidi & Camargo, 2016; Pandya et al., 2019).

In patients with a normal concentration of α-tocopherol – 25 μmol/L, its content can not increase more than 2–3 times, regardless of the amount or duration of vitamin E. The rate of oxidative metabolism of α-tocopherol has a wide range of fluctuations in different organs –the half-life period of vitamin E, determined by the introduced isotope label, ranged from 5 days in the liver and erythrocytes to 30 days in brain cells (Ingold et al., 1987).

Vitamin E deficiency is an unusual pathology because typical diets should provide a sufficient amount of it. However, malnutrition, genetic disorders and age reduce the expression of genes responsible for the synthesis of specific protein-tocopherol (α-TTP), and thus can lead to vitamin E deficiency. Premature infants with very low birth weight are prone to vitamin E deficiency. People with steatorrhea, fat absorption disorders (Kowdley et al., 1992), and hereditary diseases in which α-tocopherol-binding liver protein is defective or absent (Ouahchi et al., 1995), or who

have reduced levels of Se and Selenoproteins (Saito et al., 2015), are also likely to be deficient in vitamin E and require high doses of vitamin E supplementation.

Recently digested α-tocopherol replaces existing α-tocopherol in plasma lipoproteins, which may limit its incorporation into low-density lipoproteins (Burton et al., 1998).

Many functions of vitamin E have been studied. It is involved in the regulation of a wide range of biochemical processes that ensure the normal functioning of the body. As an effective inhibitor, especially of peroxyl radicals and singlet oxygen, it triggers the mechanism of renewal of membrane structures, participating in all three stages of antioxidant protection (Saral, 2002), and can modulate the transcription factor that regulates the expression of antioxidant enzymes (Dworski et al., 2011). It was found that the antioxidant activity of tocopherol depends on the position of the hydroxyl group on the chromanol ring (Peh et al., 2016). Tocopherol can effectively inhibit platelet coagulation and inflammation (Elisia & Kitts, 2015), as key factors influencing the course of destructive processes. These properties suggest that vitamin E can be used as a means of suppressing oxidative stress and inflammation.

It is known that in the presence of α-tocopherol stabilization of structural and functional properties of membranes occurs not only due to its antioxidant properties, but also directly in the formation of complexes with fatty acids contained in membrane phospholipids (Diplock & Lucy, 1973). Tocopherol is realized directly in the lipid bilayer of cell membranes and plasma because the hydrophobic-hydrophilic balance of the tocopherol molecule allows it to be localized among membrane lipids and interact with its lipophilic components and membrane-bound enzymes, including hydrophilic (Gomez-Fernandez et al., 1989; Llesui et al., 1995).

However, even today we are forced to state that, despite all the accumulated facts, the molecular mechanisms of action of tocopherol, its numerous biological effects, for example, for the prevention of diseases including neurological disorders, cardiovascular disease, age-related eye and skin lesions and infertility, remain to be clarified (Galli et al., 2017).

The sensitivity of membrane phospholipids to reactive oxygen species (ROS) is a result of the physicochemical properties of the membrane bilayer and the chemical reactivity of the fatty acid chains that form the membrane (Pamplona, 2011). This is due to the fact that oxygen and free radicals are more soluble in the liquid lipid bilayer than in aqueous solution. ROS free radicals, once inside biological membranes, are able to induce peroxidation of membrane lipids consisting of phospholipid residues with polyunsaturated fatty acids (Catalá, 2006; Norris et al., 2012). Polyunsaturated fatty acids (PUFAs) are formed as a result of the action of desaturases on saturated fatty acids. Being essential components of cell membranes in mammals, they are extremely sensitive to oxidation (Pamplona, 2011).

Accumulated experimental and theoretical data on model membranes with oxidized lipids showed that the cell membrane under the action of oxidative stress changes the penetration of water into the lipid bilayer due to changes in polarity (Conte et al., 2013). The resulting oxidized phospholipids alter the function and physical properties of membranes, including their fluidity, permeability, bilayer thickness, and packaging of lipids and proteins in membranes (Schnitzer et al., 2007). This is because phospholipids contain oxidized fatty acid radicals, which due to the ability to change the configuration, cause changes in the structural properties of lipid bilayers, increase their water permeability, which leads to an increase in average lipid area and, consequently, to decrease bilayer thickness (Wong-Ekkabut et al., 2007; Khandelia & Muritsen, 2009; Conte et al., 2013).

Polyunsaturated fatty acids are among the most important structural and functional elements of phospholipids in mammalian tissues. Depending on the tissue, membrane phospholipids contain 0.05–0.40% linoleic, 4–24% linolenic, 0.2–22.0% arachidonic acid (Crawford & Sinclair, 1971) in esterified form. PUFAs perform two main functions: they are important components of phospholipids of all cell membranes, and balanced fatty acid composition of phospholipids is important for adequate membrane function, because changes in their composition affect fluid and important processes such as impulse transmission, receptor function, enzymes. Depletion of the PUFA pool causes a variety of abnormalities, including infertility, ulceration, and dermatitis, and the addition of PUFA can alleviate most of these symptoms (Williard et al., 2001; Stoffel et al., 2008; Stroud et al., 2009; Roqueta-Rivera et al., 2010). The composition of PUFA in lipids is largely determined by diet, it is significantly influenced by the peculiarities of fatty acid metabolism of individuals (Grundy & Denke, 1990). Polyunsaturated fatty acids, rich in multiple unsaturated bonds, are sensitive to non-enzymatic and enzymatic oxidation. During the autooxidation of polyunsaturated fatty acids under the action of free radicals, lipid hydroperoxides are formed as primary oxidation products (Thomas, 2015). During enzymatic oxidation, phospholipase A2 cleaves phospholipids at the sn-2 position, resulting in the formation of free PUFA and lysophospholipids (Dennis et al., 2011). After release from the membrane, PUFAs can be further transformed by cyclooxygenases (COX1 or COX2) to form the H2 prostaglandin family, and free fatty acids can be converted by lipoxygenase (LOX) to form hydroperodes (Joo & Oh, 2012). Thus, the cells of all mammals contain several types of enzymes, including cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (CYP), capable of oxidizing PUFA and generating various metabolites (Gruber et al., 2015).

Arachidonic acid (AK) is synthesized in many animals (Abedi & Sahara, 2014). Endogenous AK is synthesized by the degradation of phospholipids by phospholipases such as PLA2, and it can also be synthesized by lengthening and saturating linoleic acid (C18:2) to essential fatty acid (Brash, 2001). In the form of these complexes, AK enters the cells of the whole organism. The entry of AK into cells from the extracellular environment includes the stages of dissociation of the complex, adsorption of AK on the plasma membrane, passage through the lipid bilayer and the appearance in the cytosol (Zakim, 1996).

As a fatty acid, it is present in phospholipids in the lipid bilayer and plays an important role in cell structure. Thanks to four cis-double bonds, it promotes the transformation of cell membranes, which is important for the stable functioning of cells of different tissues (Tallima & El Ridi, 2018). The question of the biological concentration of arachidonic acid becomes relevant in assessing its biological activity (Chilton et al., 1996).

The aim of the study was to check in vivo the effects of tocopherol on changes in the lipid environment of cell membranes and the associated 5-lipoxygenase oxidative metabolism of arachidonic acid, which synthesizes components of the slow-acting substance anaphylactic shock. To achieve this, it was necessary: to monitor *in vivo* the state of lipid metabolism in rat hepatocytes under the conditions of modeling of E-hypovitaminosis and to study *in vivo* the peculiarities of the oxidative metabolism of arachidonic acid under conditions of different provision of the body of rats with α-tocopherol.

Materials and methods

All the manipulations with animals were verified by the local bioethical committee of the A. V. Palladin Institute of Biochemistry of the NAS of Ukraine for the conformity to the recommendations of the European Convention for the Protection of Vertebrate Animals used for Research and Scientific Purposes (Strasbourg, 1986) and the Law of Ukraine "On Protection of Animals from Cruelty" and are ethically acceptable. The animals were killed by the displacement of cervical vertebrae under calypsol (Ketamine) anesthesia.

To create a model E of hypovitaminosis white male Wistar rats weighing 35–40 g by the method of analogues were divided into two groups: control and experimental in a ratio of 1:2. Animals in both groups were on a tocopherol-deficient E-hypovitaminosis diet for 70 days (Edwin et al., 1961). The Hypo-E diet contained a salt mixture of Osborne and Mendel (Wesson & Laurence, 1943), lard and casein, treated at 180 °C for 4 h to destroy all natural tocopherol, and all other essential vitamins and trace elements in physiological concentrations.

Animals in the control group received vitamin E in the diet at a dose of 100 mg/kg diet (10 g/animal).

On day 60, some of the animals in the experimental group (E-hypovitaminose) that did not receive vitamin E were separated from the group for correction of vitamin E. Animals in this group (correction E-hypovitaminose + vit E) received α-tocopherol at a physiological dose (1 mg α-tocopherol/100 g body weight) orally for 10 days.

Lipid extraction was performed by the method (Bligh & Dyer, 1959). An aqueous phase with calcium ions was used to reduce the adsorption of

anionic phospholipids to proteins and to more fully isolate these compounds, as recommended by Palmer (1971). Tocopherol content in liver homogenates was determined by the Brown (1952) method, on chromatography, tocopherol zones were identified according to the standard α-tocopherol (Sigma-Aldrich). Chromatographic separation of phospholipids was performed (Svetashev & Vaskovsky, 1972).

Chromatographic separation of phospholipids was made by 2D-TLC according to the method of Svetashev & Vaskovsky (1972) on silufol plates 10 x 15 cm. The obtained phospholipid extracts (10 μL of each) were applied onto activated plates and ran in a thin layer of sorbent using mixed solvent systems in two directions. In the first direction, the system chloroform-methanol-benzene-28% ammonia (65 : 30 : 10 : 6) was used, and in the second – chloroform-methanol-benzene-acetone-glacial acetic acid-water (70 : 30 : 10 : 5 : 4 : 1), respectively (Vaskovsky & Terekhova, 1979). After each direction run (and especially after the first one), the plates were dried until complete removal of solvents' residues. To detect the localization of phospholipids, the silica gel surface of the plates was treated with a developer (I_2 in hexane or 10% H_2SO_4 in methanol), the phospholipid spots obtained on the plates were quantified by a subsequent colour reaction (Dyńska-Kukulska & Ciesielski, 2012).

The content of phospholipids was measured by the amount of inorganic phosphate, which was determined spectrophotometrically at 815 nm using a molybdate reagent (Svetashev & Vaskovsky, 1972). The calibration graph was built using potassium hydrophosphate.

The lipid extract was subjected to a methylation process to determine the content of fatty acids using a modified method of Carreau and Dubaco (Carreau & Dubaco, 1978).

Quantitative analysis of methyl esters of fatty acids was performed on a Carlo Erba chromatograph (Italy) with a flame ionization detector on a glass column (2.5 m x 3 mm) filled with 10% phase SR 2300 (Silar 5CR) on Chromosorb W/HP at a programmed temperature of 140–250 °C (2 °C/min.). Standards for fatty acid methyl esters (FAME Mix, Germany) were used to calibrate the column and identify fatty acids. Measuring the area of the peaks, we calculated the percentage of fatty acids in the extract (Skoog et al., 2007).

On chromatography, cholesterol zones were identified according to the standard and eluted with benzene. Cholesterol content (Skoog et al., 2007) was determined on a Carlo Erba chromatograph (Italy)) with a dual system on a 0.5 m stainless steel column, which was filled with Chimalite W (80–100 mesh) with 1.5% liquid phase OV-1 ("Shimadzu", Japan), at the temperature of the injector and detector 250 and 270 °C, respectively, and the programmed temperature of 180–250 °C C (10 °C/min.). The internal standard for the quantification of cholesterol was β-sitosterol (Engelbrecht et al., 1974).

Study of the content of total and individual cysteine leukotrienes from the rat liver. Sample preparation, leukotriene extraction and standard preparation. The method of solid-phase extraction is described (Lindgren et al., 1983). Leukotrienes contained in the supernatant were collected with the supernatant fraction and dried in a rotary evaporator and used to determine the total content of cysteinyl leukotrienes, which were quantified by enzyme-linked immunosorbent assay (ELISA) on a reader Bio Rad Microplate Reader Model 450, and individual cysteine leukotrienes C4, D4, E4 were obtained by their separation by high performance liquid chromatography.

Cysteinyl leukotrienes contained in the blood were removed by Anderson et al. (1983)and Powell (1980) methods. The blood plasma of the test animals was mixed with 4 volumes of absolute ethanol and kept at 2–8 °C for 30 minutes. The precipitate was centrifuged at 3000 rpm for 30 minutes.

Chromatographic separation of cysteinyl leukotrienes from rats' liver and blood. Collection of individual cysteinyl leukotrienes C4, D4, E4 was performed by separating the pool of total cysteinyl leukotrienes using high performance liquid chromatography Knauer (Germany) using RPC – HPLC, column 25 * 0.5 Nucleosil ODS C18 using an eluent consisting of a mixture of acetonitrile: deionized water: acetic acid (40:60:0.1). The flow rate of the eluent was 1 mL/min. The yield of leukotrienes occurred in the following sequence; leukotriene C4, leukotriene D4 and leukotriene E4 according to the introduced calibration standard. The obtained individual cysteinyl leukotrienes: leukotriene C4, leukotriene D4 and leukotriene E4 were used for their quantification by enzyme-linked immunosorbent assay (ELISA) on Bio Rad Microplate reader model 450.

The content of total cysteinyl leukotrienes was determined by enzyme-linked immunosorbent assay (Isono et al., 1985) using BiotrakTM leukotriene C4/D4/E4 enzyme-linked immunosorbent assay system Amersham Pharmacia Biotech. The method for determining C4/D4/E4 is fast and sensitive (in the range of 0.75–48 pg), based on colour reaction. The values of the samples were calculated according to the calibration graph.

Using the methods of HPLC, it is possible to divide the pool of cysteinyl leukotrienes into individual ones, as well as to quantify the content of each of the individual cysteinyl leukotrienes C4, D4, E4. Individual cysteinyl leukotrienes were obtained by separation by high performance liquid chromatography of the peptide-leukotriene complex. The concentration of individual leukotriene C4 is calculated from the standard curve of leukotriene C4. The concentration of leukotriene D4 is calculated from the standard curve of leukotriene C4. The concentration of leukotriene E4 was determined from the curve for leukotriene C4, based on the fact that the values were obtained for the standard curve of leukotriene C4 = 10 pg/well. Cross-activity of leukotriene E4 (4–10 °C) = 70%. The concentration of leukotriene E4 = –10 x 100 = 14.3 pg/well.

The samples were checked in terms of belonging to normally distributed general populations according to the Shapiro-Wilk criterion. The parametric one-way ANOVA was used to determine reliable differences between mean values of samplings; the post-test comparison was made using the Tukey test with Boniferroni correction. In all cases the results were considered reliable on condition of the probability value P under 5% ($P < 0.05$). The results were presented as the mean ± standard error of mean (x ± SE), n – number of experiments.

Results

The study found that in the group of animals on the diet E-hypovitaminosis, the level of tocopherol in the liver was twice as low and the level of Ca^{2+} decreased by 17.1% compared to the group of control animals (Table 1). The data obtained indicate that the rats of the second group under the conditions of the experiment were in a state of e-hypovitaminosis.

Table 1
Tocopherol and cholesterol content (μmol/g of liver) in the liver of rats under different conditions of the body maintenance with vitamin E (x ± SE, n = 11)

Parameter	Control	E- hypo-vitaminosis	Correction (E- hypo-vitaminosis + vit E)
Tocopherol	53.28 ± 1.80^a	26.96 ± 1.43^b	58.49 ± 2.38^c
Cholesterol	1.52 ± 0.09^a	1.11 ± 0.13^b	2.44 ± 0.27^c

Note: different letters indicate values that differed one from another reliably within one line of the table according to the results of comparison using the Tukey test with Bonferroni correction.

In the third group of animals, in which the diet of E-hypovitaminosis was adjusted by the introduction of therapeutic doses of tocopherol, its level quickly returned to normal by 9.8%, exceeding the values in the control group. After correction of the state of E-hypovitaminosis by tocopherol, the level of calcium also increased, not reaching the control level.

Physicochemical properties of lipid components play a significant role in the formation of the lipid matrix of biomembranes. Phospholipids, cholesterol and fatty acids are structural omponents and bioregulators of membranes, they play an important role in the body's adaptive responses, regulating the permeability and fluidity of membranes.

Based on the known fact that cholesterol and phospholipids form a lipid bilayer of cell membranes, it was advisable to study their quantitative content in hepatocytes under conditions of different levels of tocopherol.

It was found that in the conditions of E-hypovitaminosis in the liver of rats the cholesterol level was reduced by 27.0% compared to the control. With tocopherol correction, its level increased by 61.0%, and the level of phospholipids in each group remained within control. The molar ratio of cholesterol/phospholipids in the conditions of E-hypovitaminosis decreased by 19.0%, and increased by 72.9% with the correction of tocopherol. It is known that changes in lipid composition, which are characterized by a

decrease in the ratio of cholesterol/phospholipids, are an unfavourable factor that leads to a decrease in the functional activity of cellular receptors and the rate of transport of metabolites and water.

Cell membranes contain a number of specific phospholipids that are necessary for the normal functioning of proteins in the membrane. The obtained data actualized further studies of the content of individual phospholipids in the membranes of hepatocytes and their components – fatty acids under conditions of different provision of the body with tocopherol are presented in Figure 1.

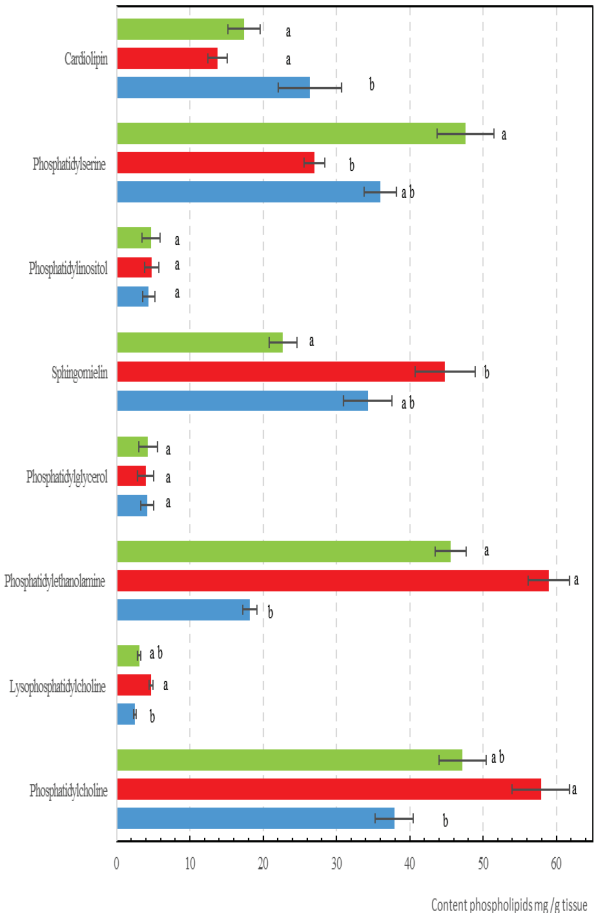

Fig. 1. The content of phospholipids in the liver of rats under different conditions of vitamin E suppl: blue colour – control group rats, red colour – E-hypovitaminosis, green colour – correction (E-hypovitaminosis + vit E) (x ± SE, n = 11, mg/g)

In our studies, it is noteworthy that in the state of E-hypovitaminosis, the group of the 8 studied individual phospholipids underwent significant changes: cardiolipin decreased by 47.7%, phosphatidylserine – by 25.0%, phosphatidylethanolamine increased by 3.24 times, lysophosphatidylcholine – by 86.9%, phosphatidylcholine – by 52.8%, and sphingomielin – by 30.6% against control group. After receiving tocopherol for the correction, the following changes were found: cardiolipin increased by 34.1%, phosphatidylserine – by 32.2% (against control), respectively. The increase of phosphatidylethanolamine by 2.52 times (against E-hypovitaminosis) was found during the correction, as well as the increase of sphingomielin by 33.8% (against control) and by 49.3% (against E-hypovitaminosis). Besides, the decrease of phosphatidylcholine by 24.5% and lysophosphatidylcholine by 23.0% against control were found, as well as by 18.5% and 34.2% against E-hypovitaminosis, respectively.

Normally, there is a constant dynamic balance in the membrane between the synthesis and decomposition of phospholipids, which changes under different conditions of physiological activity of the cell, or in the event of pathology. In our experiments it was found that with E-hypovitaminosis out of 8 studied individual phospholipids 4 of them underwent probable changes, while in their total content there were no significant fluctuations. In the body, fatty acids are in a bound state, the amount of free fatty acids is negligible. In this regard, the leading role belongs to fatty acids, which are part of phospholipids, in particular those involved in the processes of lipid polymorphism. Subsequent studies examined the effect of E-hypovitaminosis in the body on the content of fatty acids that are part of the phospholipids of the liver of rats.

According to the obtained results, different levels of tocopherol supply to the body do not significantly affect the total content of fatty acids. There is a tendency towards their reduction with E-hypovitaminosis, with the correction of tocopherol, their level returns to control as presented in Figure 2.

With E-hypovitaminosis, the total content of saturated fatty acids is within the level of control, while the content of unsaturated (palmitoleic, linoleic, linolenic, arachidonic) probably decreased compared to control. After the introduction of tocopherol during the correction there is a tendency to normalize their level.

It is well known that arachidonic acid catabolizes with the formation of biologically active eicosanoids. Therefore, given the breadth of the spectrum of biological action of eicosanoids, it was advisable to determine the metabolites of the lipoxygenase pathway of oxidative metabolism of arachidonic acid, which leads to the formation of components of the slow-acting substance of anaphylactic shock. We determine the content in the liver and blood of total and individual cysteinyl leukotrienes.

The use of ELISA provided data on the content of leukotrienes in the liver of rats which were on a diet of E-hypovitaminosis (Fig. 3). It was found that, in E-hypovitaminosis, the level of total cysteinyl leukotrienes in hepatocytes decreased by 30.3% compared to control and tended to normalize with the addition of tocopherol. The blood level of total cysteinyl leukotrienes decreased by 10.3% compared to control. During correction, their level was close to control.

To obtain data on the number of individual cysteinyl leukotrienes, the total pool of leukotrienes was separated by reverse phase high performance liquid chromatography using a Beskman ODS C18 reverse phase column and acetonitrile : methanol : deionized water system. The elution profile of individual cysteinyl leukotrienes is shown in Figure 4.

When determining the amount of individual leukotrienes, it was found that, under conditions of E-hypovitaminosis, there is a quantitative decrease in leukotrienes' C4 by 10.4%, and in D4 – by 11.5%, with constant levels of E4 in the liver (Fig. 5).

When determining the amount of individual leukotrienes, it was found that, under conditions of E-hypovitaminosis, there was a quantitative decrease in leukotrienes C4 by 10.4%, and in D4 – by 11.5%, with constant levels of E4 in the liver. With the correction by tocopherol, there was an increase in the amount of all individual cysteinyl leukotrienes. In particular, the content of leukotriene C4 increased by 25.7% compared to the E-hypovitaminosis state and exceeded the control by 12.6%. The content of leukotriene D4 increased by 2.14 times compared to the E-hypovitaminosis state and by 89.7% compared to control, and the level of leukotriene E4 increaseds by 59.5% compared to the E-hypovitaminosis state s and by 62.6% compared to control.

A probable decrease in the level of leukotriene C4 by 11.6%, D4 – 22.5%, E4 – 12.3% in the blood under conditions of hypovitaminosis has been established. Other studied indicators did not undergo significant changes in E-hypovitaminosis and its correction (Fig. 6).

Discussion

Our results coincide with the data of other researchers and indicate that under conditions of e-hypovitaminosis the body of animals is in a state of oxidative stress, impaired cell membrane function and regulation of related metabolic processes, changes in individual phospholipids (Gundermann, 2011). The composition of phospholipids in the outer and inner layers of the membranes is different. Phosphatidylcholine and sphingomyelin are charge-neutral and localized mainly in the outer lipid layer, while phosphatidylethanolamine and phosphatidylserine are localized in the inner layer (Verkleij et al., 1973). The obtained data suggest that the change in the ratio between the level of phosphatidylethanolamine and phosphatidylserine is the result of a decrease in the amount of phosphatidylserine with a simultaneous increase in phosphatidylcholine under conditions of E-hypovitaminosis.

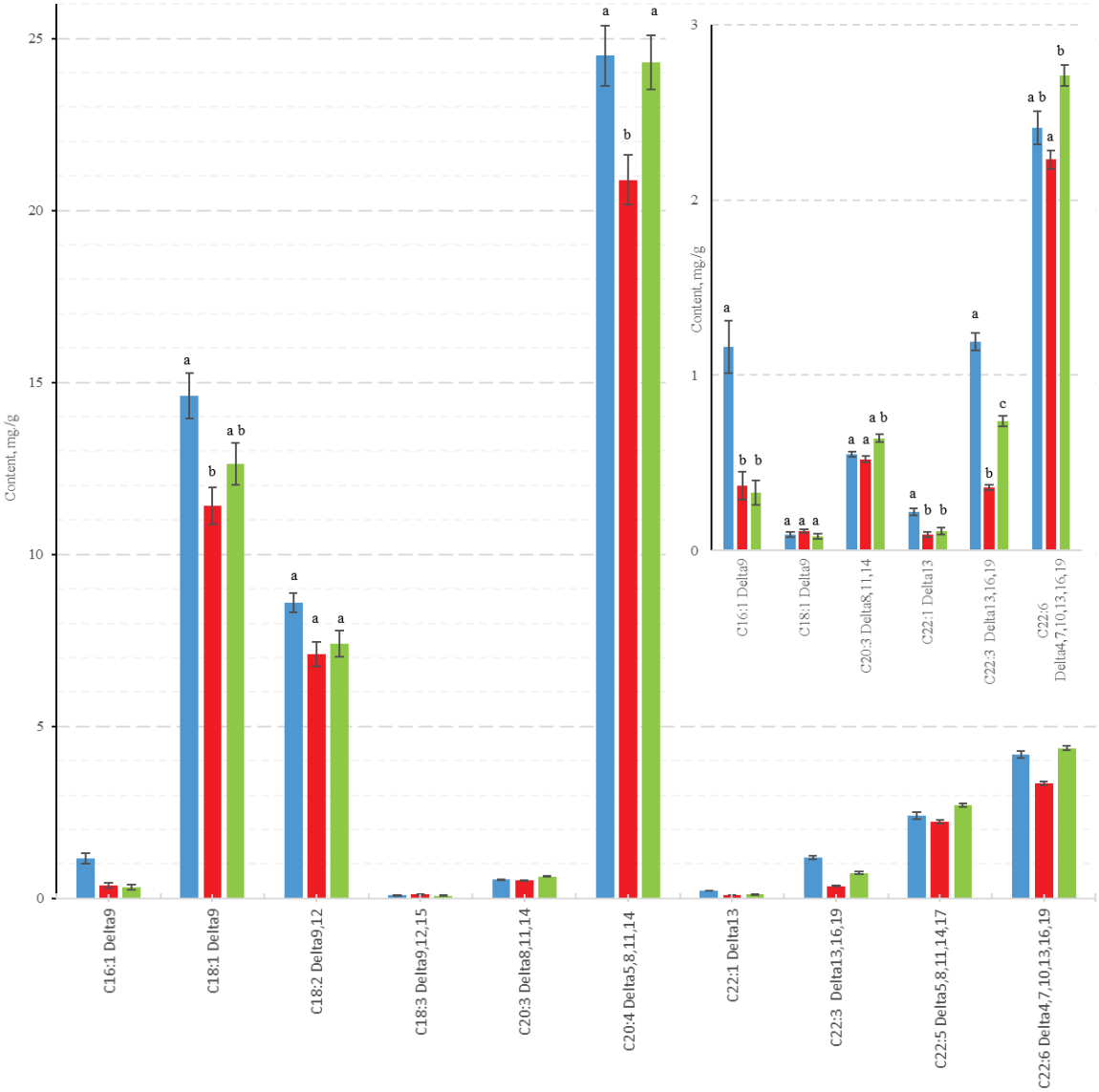

Fig. 2. The content of fatty acids in the phospholipid fraction of the liver of rats under different vitamin E supply conditions: blue colour – control group rats, red colour – E-hypovitaminosis, green colour – correction (E-hypovitaminosis + vit E) (x ± SE, n = 11, mg/g): the callout shows the data for unsaturated fatty acids with a low content in an enlarged view: see Fig. 1

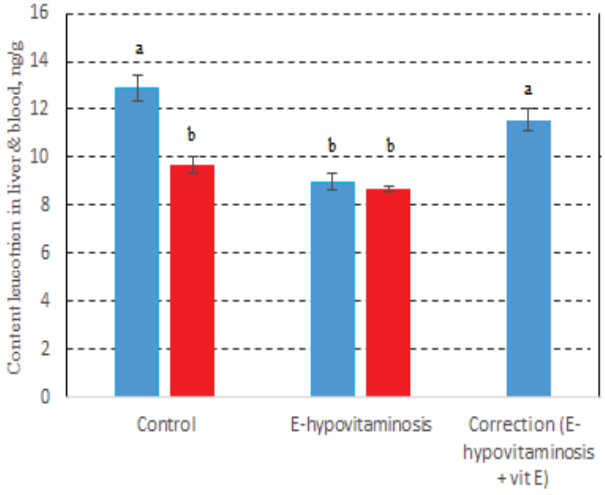

Fig. 3. Level total cysteinyl leucotriens in liver and blood under different conditions vitamin E supply: yellow colour – total cysteinyl leukotrienes (ng/mL of blood), violet colour – liver cysteinyl leukotrienes (ng/g of liver); x ± SE, n = 10

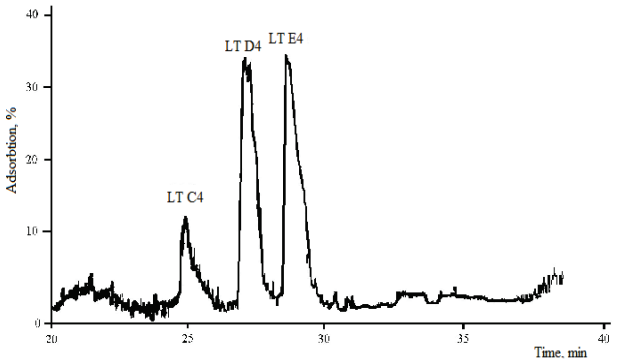

Fig. 4. Individual cysteinyl leukotrienes elution profiles by reverse phase high performance liquid chromatography (RPC – HPLC) Beskman ODS C18

The obtained changes in the content of the most common phospholipids in mammalian cells – phosphatidylcholine, sphingomyelin and phosphatidylethanolamine and phosphatidylserine in E-hypovitaminosis indicate a violation of both the outer and inner surfaces of lipid bilayer membranes. Therefore, these changes in the level of individual phospholipids in E-hypovitaminosis, changes in the ratio between the content of

individual phospholipids in the liver of animals are accompanied by dysfunction of cell membranes, regulation of related metabolic processes, changes in the synthesis and breakdown of phospholipids and play an important role (Gundermann, 2011; Parade, 2014). With the correction by α-tocopherol, these indicators tend to return to control values, so under the influence of α-tocopherol there is a stabilization of the physicochemical characteristics of the membrane, which coincides with the literature (Paradies, 2014; Hassouneh, 2018).

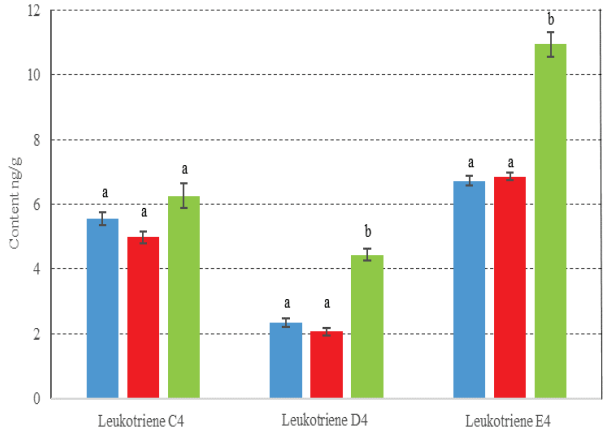

Fig. 5. The content of cysteinyl leukotrienes (C4, D4, E4) in the liver of rats under conditions of different vitamin E supply: blue colour – control group rats, red colour – E-hypovitaminosis, green colour – correction (E-hypovitaminosis + vit E): x ± SE, n = 10; see Fig. 1

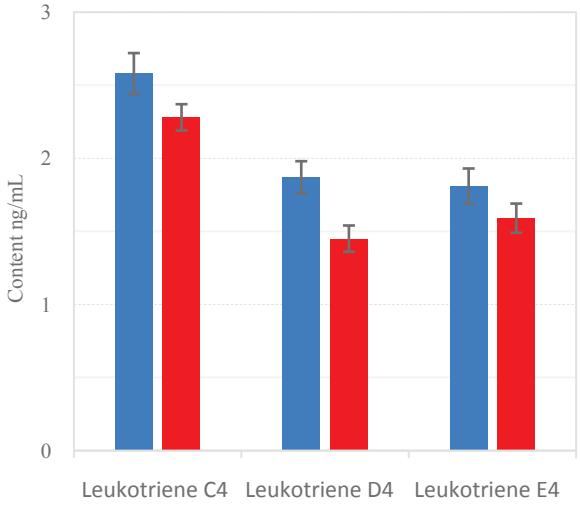

Fig. 6. The content of cysteinyl leukotrienes (C4, D4, E4) in the blood of rats under conditions vitamin E supply: blue colour – control group rats, red colour – E-hypovitaminosis, green colour – correction (E-hypovitaminosis + vit E); x ± SE, n = 10; no significant changes in the leukotriene levels

The obtained reduction of arachidonic acid content under conditions of two-fold reduction of α-tocopherol content coincides with the data of the literature, where *in vivo* and *in vitro* experiments the effect of α-tocopherol and its analogues on arachidonic acid metabolism was established. Under these conditions, the amount of linoleic and eicosatetreic acid decreases, and the level of linolenic acid, which is involved in the synthesis of arachidonic acid, increases. It is likely that changes in the level of predecessors also lead to a decrease in its level, which is observed in our studies (Raederstorff, 2015).

The correct ratio of saturated and unsaturated fatty acids in membrane phospholipids is an important factor that characterizes the properties of membranes, because the balance of cholesterol/phospholipids, as well as the composition of fatty acids and the degree of saturation, directly affect cell membrane fluidity. Similarly, their ratio can have a significant effect on cellular processes, such as the formation of lipid rafts. Lipid rafts are dynamic membrane microdomains having high levels of cholesterol and phospholipids, containing mainly saturated fatty acids involved in apoptosis or cell proliferation and unsaturated fatty acids acting as precursors for the synthesis of proinflammatory mediators – eicosanoids (Mollinedo et al., 2006; Tessaro et al., 2015). Depending on the activity of the enzyme at the bifurcation point of the metabolic pathway, the following eicosanoids are synthesized: prostaglandins, proctacyclines, thromboxanes, leukotrienes (LT), lipoxins.

It is known (Pein, 2018) that tocopherol regulates lipid peroxidation, it can affect the metabolism of arachidonic acid, which is the main substrate for the conversion of leukotrienes (LT) by oxidation of lipoxygenase. By regulating peroxidation, vitamin E can be an inhibitor of phospholipase A2 and cyclooxygenase, can affect the processes of enzymatic and non-enzymatic lipid peroxidation, in particular, the metabolism of arachidonic acid, leading to the formation of biologically active substances such as eicosanoids. The main sources of leukotriene synthesis are different types of leukocytes and some other cells. Peritoneal and alveolar macrophages play a significant role in their synthesis in inflammatory and allergic reactions. Leukotrienes are produced by all types of granulocytes, monocytes, platelets, mast cells, lung epithelium and Kupffer cells of the liver, different cell types focused on the synthesis of different leukotrienes (Keppler, 1985). The synthesis and release of leukotrienes occurs by activation of the corresponding cells by immune or other factors, their capture by hepatocytes, which have a high affinity for their absorption (Gwebu, 1980). Lungs, blood vessels and blood cells are also involved in the excretion of leukotrienes from the body (Misso et al., 2004). Once in the liver, leukotrienes are rapidly excreted in the bile.

In our studies, vitamin E deficiency caused a decrease in both the synthesis of total leukotrienes, as evidenced by their level in the blood, and the breakdown – a decrease in their content in the liver. It is important to note inhibition of the formation of all leukotrienes, especially D4. In the breakdown of leukotrienes, it is noted that E4 to a lesser extent than other cysteinyl leukotrienes, responds to a decrease in tocopherol in the body. The increase in the content of both total and individual leukotrienes in the conditions of correction confirms the property of tocopherol to affect the metabolism of leukotrienes. The obtained data indicate that the decrease in the content of α-tocopherol does not significantly change the ratio of synthesis and decomposition processes, but mainly affects their formation. Activation of leukotriene formation by α-tocopherol correction indicates the existence of mechanisms of α-tocopherol influence on arachidonic acid metabolism and leukotriene metabolism in the body, as well as of the stabilizing effect of tocopherol on the lipoxygenase pathway of oxidative metabolism of arachidonic acid.

Analysis of the data obtained during the study on the model of E-hypovitaminosis suggests that a probable decrease in tocopherol levels triggers a cascade of metabolic changes in the lipid environment of hepatocyte cell membranes. The multidirectional changes in the content of individual phospholipids that are part of the outer and inner surface of the membrane, against the background of a probable decrease in cholesterol, indicate structural changes in hepatocyte membranes caused by lower levels of tocopherol as a component of the antioxidant system. It has been established that E-hypovitaminosis reduces the level of arachidonic acid, which is part of phospholipids of cell membranes. It is very sensitive to oxidation processes, is a substrate for the synthesis of eicosanoids and causes a decrease in total cysteinyl leukotrienes in hepatocytes and blood, and accordingly, probable changes in individual cysteinyl leukotrienes C4 and D4 are the result of significant weakening of the antioxidant system. Tocopherol correction revealed a significant increase in cholesterol and phosphthidylserine levels with a decrease in lysophosphatidylcholine, phosphatidylethanolamine, normalization of arachidonic acid levels, a significant increase in the level of total and individual cysteinyl leukotrienes relative to control. Noteworthy is the significant increase in E4 relative to control and relative to E-deficiency.

Correction of the state of E-hypovitaminosis by tocopherol leads to the return of the vast majority of the studied indicators to the control levels. It is reasonable to assume that these changes are due to the restoration of the functions of the antioxidant system. Further growth of a number of phospholipids, in our opinion, may be due to the launch of compensatory

mechanisms (interconversion of phospholipids to those critical to the functioning of cell membranes, designed to replace the decline in antioxidant function) with superposition of processes of synthesis of the same critical phospholipids during complete restoration of functioning of the antioxidant system during short-term correction. Complete normalization of impaired functions requires a period of correction commensurable with the hypovitaminosis acquisition one.

Our data also indicate that due to the return to the level of control of tocopherol and unsaturated fatty acids, the sequential conversion of leukotrienes C4, D4, E4 with the participation of glutathione, which occurred by their inactivation, was activated. Similar results were obtained by Parkhomets et al. (2001), Rhee (2005), Pein (2018), when due to the return to the level of control of tocopherol and unsaturated fatty acids, including arachidonic, inhibition of tocopherol phospholipase A2 and 5-lipoxygenase in the correction of E-hypovitaminosis there was a significant increase in individual cysteinsil leukotrienes LTC4 to less active LTE4 in hepatocytes, where utilization processes predominate, which may be the result of compensatory-adaptive mechanisms aimed at utilization of cysteine, which is formed in body tissues and transported by blood. It is reasonable to assume that changes in the level of individual cysteinyl leukotrienes in the blood reflect their transformation in tissues but is inferior to the course of transformation in the liver.

Conclusion

Thus, studies in the classical model of E-hypovitaminosis in rats showed: a probable decrease in levels of tocopherol, cardiolipin cholesterol against the background of increased phosphatidylcholine, lysophosphatidylcholine and phosphatidylethanolamine. The level of oleic, linoleic and arachidonic acids, which are precursors in the synthesis of eicosanoids of different biological activity, decreased. The level of total and individual cysteinyl leukotrienes in the liver and blood decreased. Tocopherol correction of the state of E-hypovitaminosis led to the approximation of the levels of cholesterol, lysophosphatidylcholine, phosphatidyletnolamine to the control values. The level of total and individual cysteinyl leukotrienes in the liver and blood exceeded the control, which indicates the participation of tocopherol in the lipoxygenase pathway of arachidonic acid metabolism. Thus, the results indicate a potentiating effect of tocopherol on metabolic processes in the body as a whole and in individual hepatocytes. The degree of tocopherol intake allows one to influence the course of inflammatory processes associated with eicosanoids, not only through the impact on precursors, but also on the utilization of metabolites, including leukotrienes. This makes it possible to use tocopherol as an adaptogen to prevent pathology associated with the processes of enzymatic process of free radical oxydation and the development of inflammation.

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The authors declare that they have no conflict of interest.

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