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Protective Role of Intraperitoneally Administrated Vitamin E and Selenium on the Levels of Total Lipid, Total Cholesterol, and Fatty Acid Composition of Muscle and Liver Tissues in Rats

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Abstract The aim of this work was to determine the protective effects of intraperitoneally administrated vitamin E and Se on total lipid, total cholesterol, and fatty acid composition of rat liver and muscle tissues. Total lipid content of muscle tissue in Se and combination groups decreased as compared to the control group. However, the level of total lipid in the liver tissues was seen to decrease only in the combination group ($P < 0.05$). While the amount of total cholesterol in liver tissue was lower ($P < 0.05$) in the vitamin E and combination groups, the amount of total cholesterol in muscle tissue decreased ($P < 0.05$) in the combination group.

The amount of linoleic acid in muscle tissue slightly decreased ($P < 0.05$), whereas the eicosenoic and eicosatrienoic acid amounts significantly increased ($P < 0.01$, $P < 0.001$) in the vitamin E group as compared to the control group. The amounts of most fatty acid decreased ($P < 0.05$) in the combination group. The proportions of eicosenoic, eicosatrienoic, and total polyunsaturated fatty acid (PUFA) within the total fatty acid were higher ($P < 0.05$) in vitamin E group, whereas these fatty acids proportions were lower ($P < 0.05$) in the Se group. Although the proportions of palmitic, linolenic, and total saturated fatty acids were low ($P < 0.05$), oleic and total unsaturated fatty acid proportions were higher ($P < 0.05$) in the combination group than in the control group.

The amount of palmitic acid and total saturated fatty acid in liver tissue decreased ($P < 0.01$ and $P < 0.05$, respectively) in the vitamin E and combination groups. However, the amount of linoleic acid only decreased ($P < 0.05$) in the combination group. The amount of PUFA was slightly higher ($P < 0.05$) in vitamin E. The proportions of stearic acid and linoleic acid decreased ($P < 0.05$) both in the Se and combination groups. However, the proportions of eicosatrienoic, ω 6, and PUFA were slightly higher ($P < 0.05$) in the vitamin E group, but total saturated fatty acid proportion significantly decreased ($P < 0.01$) in both the vitamin E and combination groups. In conclusion, the level of total lipid and cholesterol in muscle and liver tissues were reduced by administrating vitamin E and Se together. Additionally, the fatty acid synthesis in the muscle and liver tissues was decreased by this process. However, it was observed that the protective effect of intraperitoneally administrated vitamin E was higher than Se on fatty acid composition in muscle and liver tissues. J. Cell. Biochem. 64:233–241.

Key words: intraperitoneally administrated; vitamin E; Se; liver; muscle; fatty acids; rats

Vitamin E is the name given to a number of structurally related compounds the most important of which is alpha (α)-tocopherol. The vitamin is needed for mitochondria electron transport function and prevents oxidation of various compounds including polyunsaturated (PUFA) fatty acid and vitamin A [1]. Vitamin E is an important antioxidant in biological systems [2]. It is present in lipid bilayers of biological membranes and may play a structural role there [3].

Vitamin E provides homeostasis in living cells and the mechanism by incorporating into cell membranes or entering the cells. It is readily absorbed from the intestine and transported to the liver by chylomicrons. Additionally, the absorbed vitamin E is transported through the lymphatic system as a lipoprotein complex [4]. Liver possesses an α-tocopherol binding protein that is specific for vitamin E. This protein facilitates the incorporation of α-tocopherol [2]. The

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property of tocopherol that appears to be related to most manifestations of deficiency is its inhibitory effect on the autooxidation of unsaturated fatty acid [5]. The tissues of vitamin E–deficient animals, particularly cardiac and skeletal muscle, consume oxygen more rapidly than normal tissues. α-Tocopherol does not readily undergo reversible oxidation [5]. The primary products of polyunsaturated fatty acid peroxidation in lipids are monohydroperoxides, usually referred to as lipid hydroperoxides (LOOHs) [6].

As known, the most important metabolic role of selenium in mammalian species is its function in the active site of selenoenzyme GSH-Px. This enzyme, together with super oxide dismutase (SOD) and catalase, protects cells against damages caused by free radicals and hydro- or lipoperoxides [7]. Additionally, organisms have a number of ways to reduce the accumulation of toxic lipid hydroperoxides. They may contain tocopherol or carotenoids as well as a variety of enzymes such as glutathione peroxidase, which changes the hydroperoxide group to the much less toxic hydroxy moiety. This enzyme not only allows the removal of the toxic ROOH moiety but also permits regeneration of a membrane lipid molecule through reacylation [3].

Skeletal muscle is susceptible to oxidative deterioration due to a combination of lipid oxidation catalysts and membrane lipid systems that are high in unsaturated fatty acid. To prevent or delay oxidation reactions, several endogenous antioxidant systems are found in muscle tissue [8]. These include α-tocopherol, histidine-containing dipeptides, and antioxidant enzymes such as glutathione peroxidase, super oxide dismutase, and catalase [9]. The contribution of α-tocopherol to the oxidative stability of skeletal muscle is largely influenced by diet [8]. Increasing the α-tocopherol content of rat livers by intraperitoneal administration produces liver microsomes whose resistance to lipid peroxidation is correlated with their α-tocopherol content [10].

MATERIALS AND METHODS

Animals

Thirty-two adult male Wistar rats were used in this study. At the start of the experiment the rats weighed 140–160 g and five of weeks. All rats were randomly divided into four groups and kept in a room temperature of 20°C. These animals were fed ad libidum a diet including the ingredients shown in Table I during the experiment. The first group was used as control. The second group was intraperitoneally administrated with vitamin E (dl − α-tocopheryl acetate, 10 mg/day), the third group with Se (Na_2SeO_3 0,2 mg/day), and the fourth group with vitamin E and Se (vitamin E 10 mg + Na_2SeO_3 0,2 mg/over day). This administration was done for 5 weeks. The latest dose was administrated 12 h before.

TABLE I. Diet Composition

Ingredients	%
Wheat	10
Corn	22
Barley	15
Wheat bran	8
Soybean	26
Fish flour	8
Meat-bone flour	4
Pelted	5
Salt	0.8
Vitamin mineral mix[a]	0.2

[a]Vit. A, B3, Vit. E, K_3, B1, B_2, B_6, B_{12}, nicotinamid, folic acid, biotin, Mn, Fe, Zn, Cu, I, Co, Se, antioxidant, and Ca.

Samples were prepared from animals at the end of the administrating period after overnight fasting as follows. Each experimental rat was anesthetized with ether. Then, muscle and liver tissue samples were collected. These samples were kept at −25°C until the lipid extraction and further analysis.

Lipid Extraction

The total lipids were extracted with chloroform-methanol (2:1, v/v) by the method of Folch et al. [11] as previously described [12]. Muscle and liver tissue samples were taken and homogenized with a mixer. Three grams of homogenized muscle and liver tissue samples were taken and mixed with chloroform-methanol (2:1, v/v) in a mixer. Nonlipid contaminants in lipid extracts were removed by 0.88% KCl solution. The extracts were evaporated in rotary evaporator flask and then stored at −25°C.

Determination of Total Lipid and Cholesterol

This was determined according to the method of Frings et al. [13]. Twenty microliters of each solutions from extracted lipids was treated with 200 µl of concentrated H_2SO_4 and heated in a boiling water bath for 10 min. After cooling, 10

TABLE II. Content of Total Lipid and Cholesterol in Muscle and Liver Tissues of Rats

Parameters	Control	Vitamin E	Selenium	Combination
Total lipid (muscle, g/10 g)	0.64 ± 0.03*	0.67 ± 0.05*	0.44 ± 0.02**	0.44 ± 0.01**
Total lipid (liver, g/10 g)	0.75 ± 0.01*	0.74 ± 0.01*	0.79 ± 0.04*	0.47 ± 0.03**
Total cholesterol (muscle, mg/10 g)	45.32 ± 2.52*	43.15 ± 1.21*	45.86 ± 2.82*	22.41 ± 1.35**
Total cholesterol (liver, mg/10 g)	38.44 ± 3.34*	27.24 ± 2.32*	36.01 ± 4.74*	26.16 ± 3.64**

*$P > 0.05$, not significant between groups.
**$P < 0.05$, slightly significant.

ml of phosphovanilin reagent [13] was added. The mixture was incubated at 37°C for 15 min, the samples were read at 540 nm. A good U.S. grade of olive oil (Sigma, St. Louis, MO) was used as a standard. The total lipid was calculated according to standard curve.

Total cholesterol was determined with Liebermann Burchard reaction as described by Christie [14]. The reagent was prepared by mixing acetic anhydride (60 ml) and glacial acetic acid (40 ml) at 25°C. A sample of 200 µl was treated with 5 ml of reagent and 0.5 ml of concentrated H_2SO_4 was added to it at 25°C. After 20 min the absorbance of the samples have been read at 560 nm. The calculations were performed according to standard curve, prepared by pure cholesterol standard (Merck, Darmstadt, Germany).

Fatty Acid Analysis

Fatty acids in lipid extracts were converted to methyl esters by using 2% sulfuric acid prepared as volume in methanol [12]. Fatty acid methyl ester forms were extracted three times with n-hexane. These methyl esters were separated and quantified by using gas chromatography equipped with a flame-ionization detector (600 series gas chromatography) and attached to a Unicam 4815 computing recorder. Chromatography was performed with capillary column (length 15 m, diameter 0.32 mm) using nitrogen as carrier gas (flow rate 2.5 ml/min). The temperatures of the column, detector, and injection port were 185, 200, and 200°C, respectively. Retention times and peak areas were automatically computed by an Atiunicam recorder. Identification of the individual methyl esters was performed by frequent comparison with authentic standard mixtures analyzed under the same conditions.

Statistical Analysis

The collected values from groups were reported as mean ± SE. Statistical analysis was performed using SPSS 6.0 Software. The variance analysis (ANOVA) and LSD test were used for comparison between groups.

RESULTS

Total Lipid and Cholesterol (g/10 Wet Tissue)

Total lipid content of muscle tissue slightly decreased ($P < 0.05$) in the selenium and combination groups as compared to control, while the total lipid content of liver tissues only decreased ($P < 0.05$) in the combination group. As the total cholesterol content of liver tissues was slightly decreased ($P < 0.05$) in the vitamin E and combination groups, the total amount of cholesterol in muscle tissues only decreased ($P < 0.05$) in the combination group (see Table II).

Fatty Acid Content of Muscle and Liver Tissue Lipids (mg/10 g, Wet Tissue)

In muscle tissues, the amount of palmitic acid, total fatty acid, saturated and unsaturated fatty acids, total omega (ω)-6, and total polyunsaturated fatty acids (PUFA) were found to be lower ($P < 0.05$) in the combination group than in other groups. The amount of oleic acid slightly decreased ($P < 0.05$) in selenium and combination groups as compared to control. But the amount of linoleic acid was lower ($P < 0.05$) in the vitamin E and combination groups. However, the amount of linolenic, eicosatrienoic acids and total ω-3 were significantly higher ($P < 0.001$) in the vitamin E group than other groups. In addition, the amount of eicosenoic acid was significantly high ($P < 0.01$) in the vitamin E group (see Table III).

In liver tissues, while the amount of palmitic acid decreased significantly ($P < 0.01$) in the vitamin E and combination groups, it was seen to slightly decrease ($P < 0.05$) in the selenium group as compared to control. The amount of linoleic acid slightly decreased ($P < 0.05$) in the combination group as compared to other groups.

TABLE III. Amounts of Fatty Acids in Muscle Tissues (mg/10 g, wet tissue)*

Fatty acids	Control	Vitamin E	Selenium	Combination
16:0	124.39 ± 19.40*	132.57 ± 10.88*	119.31 ± 16.80*	74.74 ± 8.20**
18:1	141.21 ± 18.46*	147.15 ± 15.89*	139.17 ± 16.74*	89.31 ± 13.40**
18:2	122.62 ± 14.10*	114.38 ± 13.49**	121.98 ± 16.25*	76.58 ± 9.22**
18:3	8.94 ± 1.34*	18.45 ± 4.85[d]	7.66 ± 0.66*	3.84 ± 0.35**
20:1	3.99 ± 0.47	8.07 ± 3.13[c]	1.55 ± 0.49**	1.87 ± 0.14**
20:3	9.87 ± 0.49*	25.61 ± 10.57[d]	9.79 ± 0.57*	7.94 ± 0.50*
20:4	2.31 ± 0.38*	3.13 ± 0.22**	2.86 ± 0.30*	2.88 ± 0.04*
20:5	1.66 ± 2.98	1.51 ± 0.03**	0.81 ± 0.27[c]	0.89 ± 0.14[c]
Σ	414.80 ± 54.95*	451.07 ± 20.72*	399.33 ± 47.55*	260.01 ± 30.75**
Σ Saturated	124.39 ± 19.40*	132.84 ± 10.32*	119.31 ± 16.98*	72.74 ± 8.20**
Σ Unsaturated	290.67 ± 34.74*	317.93 ± 10.28*	280.09 ± 30.95*	187.32 ± 22.56**
Σ ω-3	10.55 ± 1.60*	19.96 ± 4.84*	8.47 ± 0.39*	4.74 ± 0.44**
Σ ω-6	133.40 ± 14.36*	144.35 ± 5.08*	130.92 ± 14.32*	87.39 ± 9.29**
Σ PUFA	145.35 ± 15.91*	163.09 ± 4.75*	139.39 ± 14.70*	92.13 ± 9.42**
Σ MUFA	151.16 ± 18.80*	154.89 ± 12.71*	140.72 ± 16.34*	95.18 ± 13.56**

*$P > 0.05$, not significant between groups.
**$P < 0.05$, slightly significant.
[c]$P < 0.01$, more significant.
[d]$P < 0.001$, most significant.

TABLE IV. Proportions of Fatty Acids in Rat Muscle Tissues (%)

Fatty acids	Control	Vitamin E	Selenium	Combination
16:0	29.54 ± 1.12*	29.22 ± 1.59*	29.58 ± 1.37*	28.06 ± 0.25**
18:1	34.04 ± 0.02*	32.16 ± 3.73*	34.79 ± 1.07*	35.48 ± 1.80**
18:2	29.82 ± 0.91*	29.91 ± 3.09*	30.31 ± 0.67*	29.40 ± 1.38*
18:3	3.84 ± 2.11*	4.39 ± 2.16*	1.96 ± 0.12**	1.57 ± 0.37**
20:1	0.98 ± 0.14*	1.98 ± 1.33**	0.47 ± 0.24*	0.74 ± 0.06*
20:3	2.59 ± 0.59*	6.32 ± 4.47**	2.54 ± 0.25*	3.25 ± 0.59*
20:4	0.55 ± 0.05*	0.70 ± 0.06*	0.80 ± 0.23*	0.88 ± 0.37*
20:5	0.37 ± 0.05*	0.24 ± 0.11*	0.18 ± 0.17**	0.34 ± 0.03*
Σ Saturated	29.55 ± 1.12*	29.88 ± 2.09*	29.58 ± 1.37*	28.06 ± 0.25**
Σ Unsaturated	70.51 ± 1.13*	70.72 ± 1.622*	70.51 ± 1.13*	71.96 ± 0.25**
Σ ω 3	2.52 ± 0.16*	4.73 ± 2.18**	2.22 ± 0.27*	1.91 ± 0.38*
Σ ω 6	32.96 ± 1.16*	32.17 ± 1.71*	32.88 ± 0.47*	33.83 ± 1.70*
Σ PUFA	35.48 ± 1.08*	36.66 ± 3.83*	35.19 ± 0.74*	35.74 ± 1.53*
Σ MUFA	35.02 ± 0.12*	34.07 ± 2.47*	35.26 ± 1.11*	36.22 ± 1.75*

*$P > 0.05$, not significant between groups.
**$P < 0.05$, slightly significant.

The amounts of stearic, oleic, eicasatrienoic, and eicosapentaenoic acids were not significant ($P > 0.05$) between groups. Additionally, the amounts of total fatty acid, unsaturated fatty acid, ω-3, ω-6, and monounsaturated fatty acids (MUFA) were not significant ($P > 0.05$) between groups. But the amount of total ω-3 fatty acid was slightly greater ($P < 0.05$) in the vitamin E group than in other groups (see Table V).

Fatty Acid Proportions of Muscle and Liver Tissue Lipids (%)

In muscle tissue, the proportion of arachidonic, eicosapentaenoic, linoleic, linolenic, palmitic acids and total ω-6, total PUFA, and saturated and unsaturated fatty acid did not vary in the vitamin E group by comparison to control. However, eicosenoic, eicosatrienoic, oleic, and total ω-3 fatty acids were slightly high ($P < 0.05$) in the vitamin E group. The proportion of linolenic and eicosapentaenoic acids slightly decreased ($P < 0.05$) in the selenium group as compared to control. However, there was no change in the proportion of other fatty acids between groups ($P > 0.05$). As the proportion of palmitic, linolenic, and total saturated fatty acids were slightly decreased ($P < 0.05$) in the combination group according to the control

TABLE V. Amounts of Fatty Acids in Rat Liver Tissues (mg/10 g)

Fatty acids	Control	Vitamin E	Selenium	Combination
16:0	53.36 ± 5.80	41.71 ± 3.37***	50.09 ± 1.13**	37.70 ± 0.70***
18:0	47.29 ± 5.04*	46.39 ± 2.79*	49.89 ± 1.19*	41.01 ± 2.95*
18:1	36.70 ± 3.72*	30.01 ± 1.42*	35.40 ± 0.87*	32.48 ± 3.20*
18:2	47.42 ± 5.04*	45.62 ± 2.39*	47.93 ± 2.26*	34.85 ± 1.91**
20:3	39.67 ± 4.87*	47.63 ± 3.35*	43.03 ± 0.96*	43.89 ± 2.01*
20:5	3.45 ± 0.54*	3.71 ± 0.06*	3.57 ± 0.41*	3.63 ± 0.52*
Σ	227.88 ± 24.51*	211.99 ± 10.24*	229.93 ± 4.53*	191.54 ± 9.69**
Σ Saturated	100.69 ± 10.77*	88.10 ± 4.45*	99.97 ± 2.25*	78.71 ± 3.64**
Σ Unsaturated	127.24 ± 13.73*	123.91 ± 6.23*	129.93 ± 3.41*	112.85 ± 6.08**
Σ ω 3	3.45 ± 0.54*	3.71 ± 0.06*	3.57 ± 0.41*	1.63 ± 0.51**
Σ ω 6	87.09 ± 9.71*	90.18 ± 5.24*	90.96 ± 3.18*	78.74 ± 3.78**
Σ PUFA	90.54 ± 10.05*	98.63 ± 4.34**	94.53 ± 3.59*	80.37 ± 3.41*
Σ MUFA	36.70 ± 1.72*	30.01 ± 1.42**	35.40 ± 0.87*	32.48 ± 3.11**

*$P > 0.05$, not significant between groups.
**$P < 0.05$, slightly significant.
***$P < 0.01$, more significant.

TABLE VI. Proportions of Fatty Acids in Rat Liver Tissues (%)

Fatty acids	Control	Vitamin E	Selenium	Combination
16:0	23.44 ± 0.29*	19.73 ± 2.18*	21.80 ± 0.73*	19.84 ± 0.97*
18:0	20.75 ± 0.52*	21.85 ± 1.06*	15.30 ± 6.60**	21.30 ± 0.71*
18:1	16.18 ± 0.53*	14.19 ± 0.58*	15.41 ± 0.58*	16.80 ± 1.28*
18:2	20.84 ± 0.10*	20.10 ± 0.62*	20.81 ± 1.08*	18.20 ± 0.96**
20:3	13.97 ± 3.08	22.38 ± 1.26**	18.73 ± 0.61**	22.94 ± 0.17**
20:5	1.50 ± 0.27*	1.77 ± 0.10*	1.54 ± 0.23*	0.92 ± 0.46*
Σ Saturated	44.80 ± 0.56***	41.58 ± 1.12*	43.50 ± 1.15***	41.14 ± 0.42*
Σ Unsaturated	55.76 ± 0.32*	58.43 ± 1.13**	56.49 ± 1.14*	58.87 ± 0.42**
Σ ω 3	1.50 ± 0.27*	1.77 ± 0.10*	1.54 ± 0.23*	0.92 ± 0.46*
Σ ω 6	38.08 ± 0.28*	42.25 ± 1.79**	39.54 ± 1.53*	43.55 ± 2.13**
Σ PUFA	39.58 ± 0.30*	44.22 ± 1.71**	41.08 ± 1.72*	42.07 ± 1.30
Σ MUFA	16.18 ± 0.53*	14.19 ± 0.58*	15.41 ± 0.58*	16.80 ± 1.28*

*$P > 0.05$, not significant between groups.
**$P < 0.05$, slightly significant.
***$P < 0.01$, more significant.

group, the proportion of oleic and total unsaturated fatty acids were slightly high ($P < 0.05$) in the same group (see Table IV).

In liver tissues, as the proportion of palmitic and eicasapentaenoic acids did not differ between groups, the proportion of stearic acid slightly decreased ($P < 0.05$) in the selenium group. In addition, the proportion of oleic acid did not vary between groups, whereas the proportion of linoleic acid was lower ($P < 0.05$) in the combination group than other groups. However, the proportion of eicosatrienoic acid was significantly higher ($P < 0.01$, $P < 0.05$) in the administration groups than control (see Table VI). As the proportion of unsaturated and total ω-6 fatty acid slightly increased ($P < 0.05$) in the vitamin E and combination groups as compared to control, the proportion of saturated fatty acid significantly decreased ($P < 0.05$) in the same groups. The proportion of PUFA was also slightly higher ($P < 0.05$) in the vitamin E group than other groups. However, the proportion of total MUFA was not significant ($P > 0.05$) between groups (see Table VI).

DISCUSSION

In the present data, it was found that the total lipid content of liver tissue was between 0.47 ± 0.03–0.79 ± 0.04 g/10 g and that total cholesterol content was 26.16 ± 3.64–38.44 ± 3.34 mg/10 g wet tissue (see Table II). It has been stated that the content of total lipid in liver tissue of an adult rat was 60 g/1 kg fresh weight tissue and total cholesterol content was

3 g/1 kg wet tissue [15]. The liver tissue of mammal was generally found to be quite rich in lipid [1] and fat represents about 50% of the total calories available for metabolic purposes with a range of 69% in rats [16].

While the total lipid content of liver tissue slightly decreased ($P < 0.05$) in the combination group, there was no difference between control and other groups ($P > 0.05$) at the end of the administration period. Gurr and Harwood [3] have reported that the liver of mammals can accommodate fat in the form of small globules but only in the short term. The excessive accumulation of fat in mammalian liver is a pathological condition.

A greater accumulation of fat in liver tissue is not suitable in health conditions [3]. The total lipid content of liver tissue has been reduced by the vitamin E and selenium supply. In addition, the total lipid content has reduced by the alone of selenium, together of vitamin E, and selenium in muscle tissue (see Table II). A decrease of total lipid content in the selenium and combination groups may be due to administration selenium and vitamin E. Karsai and Gaal [17] have suggested that the supply of vitamin A, vitamin E, and Se plays an important role in prophylaxis of fatty liver in healthy cows.

The results of gas chromatography analysis showed that palmitic (16:0), oleic (18:1), and linoleic (18:2) acids formed a great part of fatty acid content in muscle tissue. Additionally linolenic (18:3) and eicosatrienoic (20:3) acids were found to be partially greater than long chain fatty acids such as eicosapentaenoic (20:5) and arachidonic (20:4) (see Tables III, IV). The palmitic, stearic (18:0), oleic, linoleic, and eicosatrienoic acids were major, but eicosapentaenoic acid minor, proportions of fatty acid content in rat liver tissues (see Tables V, VI).

Gunstone et al. [1] have stated that the total fatty acids of land animal liver contain 30–40% of the total acids as saturated (mainly palmitic and stearic). In our data, it was found that the proportion of total saturated fatty acid to be 41.14 ± 0.42–44.80 ± 0.56% in the liver tissues of rats (see Tables V, VI). The proportion of total saturated fatty acid in muscle tissue was less than in liver tissues, but the proportion of total unsaturated fatty acid in muscle tissue was less than in liver tissues, but the proportion of total unsaturated fatty acid in muscle was higher than in liver (see Tables IV, VI). Chan and Decker [8] have reported that the composition of unsaturated fatty acids was found to be higher in muscle tissue membrane. Although the palmitic acid existed within the fatty acid composition of both tissues, no stearic acid was present in the muscle tissue of rats (see Tables III, V).

Mathews and van Holde [18] have stated that the major organs in fuel metabolism are brain, muscle, liver, adipose tissue, and heart. Muscle can utilize a variety of fuels: glucose, fatty acids, or keton bodies. Skeletal muscle needs energy and fuels and consumes it in line with its wide variations in activity. In resting muscle, fatty acids represent the major energy source; glucose is the primary source during exertion.

A major metabolic role of liver is the synthesis of fuel components for utilization by other organs. The liver is a major site for fatty acid synthesis [18]. However, findings of less fatty acid in liver than muscle tissue are believed to be due to the fact that liver fatty acid may transfer as fuel and structural molecules to other tissue following their production in tissue (see Tables III, V). Additionally, liver obtains much of its fatty acid complement from circulating fatty acid; thus, liver fatty acid composition may be influenced greatly by plasma lipid fatty acid composition [19].

Within the fatty acid composition of liver and muscle tissues, the ω-6 or n-6 fatty acids have been found to be greater than ω-3 or n-3 fatty acid (see Tables III, V). Although the ω-6 fatty acid content of muscle tissue was higher than liver, the proportion of ω-6 fatty acid within total fatty acid in liver was higher than muscle (see Tables III–VI).

Gurr and Harwood [3] reported that organs and tissues performing functions such as chemical processing (e.g., liver) and mechanical work (e.g., muscle) tend to have membranes in which the ω-6 fatty acids predominate with arachidonic acid as the major component. In our results, the ω-6 fatty acids have been found to be dominant within the unsaturated fatty acid composition of muscle and liver tissues (see Tables IV, VI). Although the amount of arachidonic acid was lower in muscle tissue, this fatty acid was not detected within the fatty acid composition of liver. In contrast the amount of eicosatrienoic acid (n-6) was found to be higher in liver tissue than muscle tissue.

In the synthesis of unsaturated fatty acids, the most likely alternative pathway is the one beginning with oleic acid and this generates a

20-carbon acid with the structure all-cis-5, 8, 11, eicosatrienoic acid in place of arachidonic acid [3]. This acid is not normally present in tissues in more than minute amounts and its accumulation provides a biochemical diagnosis of the occurrence and extent of essential fatty acid deficiency [3, 14]. Membranes of liver mitochondria from essential fatty acid deficient animals have smaller proportions of linoleic and arachidonic acids and larger proportions oleic acid and eicosatrienoic acid than those of healthy animals [3]. Experiments with laboratory animals have shown that even very small quantities of linoleic acid are sufficient to protect the animal from essential fatty acid deficiency as long as the remainder of the diet is low in fat [3].

The level of PUFA in liver tissues was found to be higher than in muscle. The higher PUFA proportion in liver may be due to the synthesis of these fatty acid by liver. However, the level of linoleic acid (18:2, ω-6) has been found to be greater within the ω-6 fatty acid composition of both tissues. But the proportion of this fatty acid was higher within the fatty acid composition of muscle tissue than liver. Christie [14] and Rule et al. [20] have reported linoleic acid (18:2, ω-6) and linolenic acid (18:3, ω-3) need to be supplied by diet, and arachidonic acid (20:4, ω-6) and eicospentaenoic acid (20:5, ω-3) would be synthesized from 18:2 and 18:3. Christie [21] reported that in muscle cell membranes phospholipids contain high proportions of 18:2 and 20:4 and may actually serve as a storage function for these fatty acids.

The amounts of palmitic, oleic, and total fatty acid, and saturated, unsaturated ω-6, ω-3, PUFA, and MUFA within the fatty acid content of muscle tissue slightly decreased ($P < 0.05$) in the combination group at the end of the administration period (see Table III). Although the amounts of eicosenoic, eicosatrienoic, and arachidonic acids and unsaturated fatty acids increased in vitamin E ($P < 0.01$, $P < 0.001$, $P < 0.05$, respectively), the amount of linoleic acid decreased ($P < 0.05$) in the vitamin E and combination groups. While the eicosapentaenoic acid slightly decreased ($P < 0.05$) in the vitamin E group, it was significantly low ($P < 0.01$) in other administration groups as compared to control (see Table III). Increase of eicosatrienoic (20:3, ω-6) and arachidonic acids (20:4, ω-6) in the vitamin E group show that these fatty acids may be synthesized from linoleic acid. Because the amount of linoleic acid decreased in the vitamin E group. These results show the protective effect of vitamin E on the metabolism of these unsaturated fatty acids.

It has been reported that peroxidation of unsaturated fatty acid in the endoplasmic reticulum of muscle results in release of lysosomal hydrolases, thereby causing muscular dystrophy. All manifestations of vitamin E deficiency appear to be secondary to the uninhibited peroxidation of polyunsaturated fatty acids [5]. Increasing the α-tocopherol content of rat livers by intraperitoneal administration produces liver microsomes whose resistance to lipid peroxidation is correlated with their α-tocopherol content [10].

In the vitamin E group, the increase in the unsaturated fatty acids such as eicosenoic, eicosatrienoic, linolenic, and arachidonic acids within the fatty acid content of muscle tissue may be due to the protective effect of vitamin E on the metabolism of these fatty acids. In addition, the proportions of eicosenoic, eicosatrienoic, and total ω-3 were higher in the vitamin E group (see Table IV). Increase of these fatty acid proportions in the vitamin E group supports the preceding hypothesis (see Tables III, IV). However, the proportion of linoleic and eicosapentaenoic acids was decreased in the selenium group. In the selenium group, the decrease of these fatty acids in muscle tissue shows that the administration of selenium was less effectual than vitamin E. In contrast, the decrease of the proportions of palmitic and total saturated fatty acid and the increase of oleic and total unsaturated fatty acid proportions in the combination group may be due to the administered vitamin E (see Table IV).

Vitamin E and Se have synergistically many functions in cells [22]. One of their most important characteristics is their intracellular and intercellular antioxidant property. Therefore these properties avoid the oxidation of polyunsaturated fatty acid in cell membranes and protect them during the metabolism [23–25].

The administration of selenium has a less protective effect than vitamin E on the fatty acid content and composition of muscle tissue. Smith et al. [5] have reported that the selenium-containing protein is present in muscles of lambs and calves. A less effective selenium administration may be due to the little concentration of glutathione peroxidase in rat muscle tissue. In liver tissue, the amount of palmitic

acid significantly decreased ($P < 0.01$); however, linoleic acid and total saturated fatty acid slightly decreased ($P < 0.05$) in the combination group. On the other hand, the amount of total PUFA increased slightly ($P < 0.05$) in the vitamin E group as compared to control (see Table V).

Increase of total PUFA amount in the vitamin E group may be due to the protective effect of vitamin E on the metabolism of polyunsaturated fatty acids. Also, the proportions of PUFA and ω-6 in the vitamin E group were higher than the control group. In addition, the proportion of ω-6 increased in the combination group (see Table II, V). In contrast, the amount of total cholesterol in liver tissue was lower in the vitamin E and combination groups. The reduction in the amount of cholesterol in these groups may due to the inhibitor effect of polyunsaturated fatty acids. The increase of eicosatrienoic, total unsaturated fatty acid, and total ω-6 proportions in the vitamin E and combination groups support the case. Additionally, the proportion of total PUFA was high in the vitamin E group (see Table VI).

Chautan et al. [26] have reported a lowering effect of n-3 PUFA on plasma cholesterol level and biliary cholesterol secretion in animals fed different oil-enriched diets. Also, the investigators observed little decrease in plasma lipid levels in animals fed corn oil. Conversely, in rats fed salmon oil, a marked decrease in plasma cholesterol level was noted. Some authors have claimed that vegetable n-6 polyunsaturated fatty acids enhance biliary cholesterol secretion.

It has been shown that the administration of vitamin E and Se has a protective effect on the fatty composition in the organs of body, especially unsaturated fatty acids (n-3 and n-6). The tissues that protect these fatty acids are believed to cause the decrease in the synthesis of total lipid and cholesterol.

However, our results show that vitamin E administration was more effective on liver tissue of unsaturated fatty acid than selenium. Normal rat liver cells in regenerating liver show the reduction of the rates of peroxidation and the increase of α-tocopherol content [9]. It has been reported that the amount of lipid peroxides in liver increases with a decreasing amount of vitamin E in serum and liver [6]. This has also been confirmed by measuring the amount of lipid peroxides in liver by thiobarbituric acid-reactive substances [2, 6, 27].

In conclusion, the level of total lipid and cholesterol in muscle and liver tissues was reduced by administrating vitamin E and Se together. Additionally, the fatty acid synthesis in muscle and liver tissues was decreased by this process. However, it was observed that the protective effect of intraperitoneally administrated vitamin E was greater than Se on the fatty acid composition of liver and muscle tissues.

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