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EFFECT OF ANTIOXIDANT ON HEPATIC FATTY ACID PATTERN IN VITAMIN E-DEFICIENT RATS

HAFEZ, S. A.[1] AND S. A. ISMAEL[2]

1 Special Food & Nutrition Department, Food Technology Research Institute, ARC

2 Biochemistry Department, Faculty of Agriculture, Cairo University

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Abstract

The present investigation aims to find a proper answer whether can the antioxidant effect of vitamin E be substituted by the antioxidant effect of high levels of vitamin C, A or selenium ion using the fatty acid pattern of liver as an indicator for lipid peroxidation?. Rats were fed o a standard diet containing vitamin E stripped con1 or cotton seed oils for 49 days. Excess amounts of vitamin A (0.45 g/day), vitamin C (7.5 mg/day), sodium selenite (0.1 mg/day), or water were daily administered by a stomach tube. Normal rats fed on diet containing corn or cotton seed oil for 6 weeks were used as control groups. Fatty acid composition of the liver was determined by gas-liquid chromatography technique. There was . significant decrease in $C_{18:2}$ in vitamin E-deficient corn oil group and it was completely absent in vitamin E-deficient cotton seed oil group with increase the $C_{18:0}$ and $C_{18:1}$. Administration of vitamin A in the presence of vitamin E-stripped cotton seed oil, gave satisfactory results by increasing of $C_{18:2}$ and decreasing of $C_{18:0}$ and $C_{18:1}$. While, in the presence of vitamin E-stripped corn oil, the autoxidation of $C_{18:2}$ was increased, therefore short chain fatty acids were present relatively in large amounts compared to its control group (corn oil group). Administration of vitamin C in the presence of vitamin E-stripped corn oil significantly increased hepatic stearic acid from 5.1% to 46.6% and oleic acid from 3.7% to 11.1%. Administration of selenite ion to the sane group moderately increased stearic, oleic and linoleic acids. Selenite ion was the best additive to vitamin E-stripped corn oil and it improved the pattern of fatty acids containing eighteen carbon atoms ($C_{18:0}$, $C_{18:1}$, $C_{18:2}$). In the case of vitamin E-deficient rats fed on cotton seed oil free from vitamin E, administration of vitamin C or selenite ion gave the best results, in which, the fatty acid patterns became similar to its control group (cotton seed oil groups).

Key words: Vitamin E, A, C antioxidant, hepatic fatty acid pattern. lipid peroxidation, selenite ion.

INTRODUCTION

The role of dietary antioxidants in health and disease has attracted increasing attention in the last few years. Numerous trials are currently proceeded to ascertain the potential benefits of antioxidant supplements to patients with various diseases (1, 2). Substantiál evidence demonstrates a multitude of interactions among antioxygenic sub-

stances in living cells, hence, investigators have used multiple antioxidant supplements in food (3, 4). Recently, it was reported that the combination of vitamin E, vitamin A and selenium provided greater protection than that happened by either of them alone (5, 6).

There is an increasing evidence that, as a result of infection, action of toxic chemicals or radiation, the body may be continuously exposed to the damaging action of free radicals (7, 8). Previous results have suggested that zinc and calcium may be involved in limiting the formation of the highly reactive species (9, 10). It was reported that natural organic antioxidants may play an important role for reducing this effect. Many results illustrated the quantitative and qualitative differences in the free radical activity after addition of vitamin E, C, β-carotene or glutathione to the living organism (11, 12, 13, 14).

It has been shown, in animals that the dietary requirement of vitamin E increases when the intake of polyunsaturated fatty acids (PUFA) increases (15, 6). An experimental study on humans shoved that a two fold increase in PUFA intake. resulted in a gradual decrease in plasma ∝-tocopherol (16). The relationship between dietary ∝-tocopherol and PUFA in diets is not clear, since the primary sources of PUFA in food supply are olive, sunflower, soybean, cotton seed, or corn oils, which are also rich in ∝- and ɣ-tocopherols. Therefore, the increase in PUFA intake is automatically accompanied by additional vitamin E Furthermore, about one half of the vegetable oils consumed are in the form of hydrogenated shortenings or margarine in which the ratio of vitamin E to PUFA is increased over that of the unhydrogenated oils, since the total amounts of PUFA are usually decreased by hydrogenation. A present evidence indicates that the suggested ratio of dietary tocopherols: PUFA is 0.6 (17). This ratio is higher than the recommended ratio in the United States (0.4), as reported by Bieri and Evarts (18).

The individuals usually consume excessive quantities of vegetable oils (more than 20 g/day), if they abruptly terminate this intake, a state of relative deficiency of vitamin E can develop, since ∝-tocopherol is lost from tissues faster than polyunsaturated fatty acids (19). In such case, supplemented vitamin may be advisable for an extended period after discontinuance of the high PUFA intake (20). It is well established that the requirement of vitamin E is related to the fatty acid composition of tissue lipids. Unfortunately, the complex relationship between dietary lipid composition and tissue lipid composition are not understood or appreciated by many investigators.

The present investigation aims at studying the effect of vitamin E-stripped cotton seed oil and vitamin E-stripped corn oil, as a sole source of lipid in diet, with different natural antioxidants (vitamin E, C, A or selenium) on the fatty acid pattern of liver in vitamin E-deficient rats. The main idea of this research is to find a proper answer for this question, can antioxidant effect of vitamin E be substituted by the antioxidant effect of high levels of vitamin C, A or selenium? . Fatty acid pattern of liver was used as an indicator for lipid peroxidation.

MATERIALS AND METHODS

Refined corn and cotton seed oils, free from synthetic antioxidants, were obtained directly from Cairo factory for oils and soaps production. Different types of antioxidants including vitamin E, C and A were obtained from Sigma Company (Cairo office). Sodium selenite (Na_2 SeO_3) used as a source of selenium, was obtained from El-Nasr company for chemical and pharmacological industries production, pure grade 89%. Experimental animals were purchased from egyptian organization for biological products & vaccines.

Preparation of tocopherol-Stripped oil :

It was prepared according to the method described by Witting and Horwitt (21).

Determination of tocopherol :

Tocopherol was determined according to the method of Vatassery and Smith (22) using a high-performance liquid chromatography (HPLC) technique.

Experimental animals :

One hundred rats (Sprague Dawley) weighing 150 170 g each were kept in stainless steel cages, the housing was air conditioned by an artificial air circulation. The animals were caged in ten groups, each of ten rats. All rats were acclimated for one week prior to the experiment. During this time, the standard diet AOAC (23) and water were available ad-libitum.

Experimental animal design:

Eighty rats were divided into two main groups. The first group received vitamin E-stripped corn oil instead of the lipid fraction in the basal diet and the second one received vitamin E-stripped cotton seed oil.

Rats fed either vitamin E-stripped corn oil (40 animals) or E-stripped cotton seed oil (40 animals) were caged in four subgroups (each of 10 animals) which were orally and daily administered with excess amount of vitamin A (0.45 g/day), vitamin C (7.5 mg/day), sodium selenite (0.1 mg/day) or water (control) by a stomach tube. Twenty rats (positive controls), in two groups, received the standard diet containing either corn oil (10 rats) or cotton seed oil (10 rats). After the experimental period (42 days), the animals were sacrificed and livers were removed and analyzed for determination of fatty acid composition.

Gas-liquid chromatographic analysis:

An accurate weight of liver was taken and extracted in a soxhelt apparatus by methanol-chloroform (2:1) for 16 hours. The methanol-chloroform layer was evaporated to dryness in a water bath. Saponification, extract and methylation of free fatty acids were carried as described by Getz and Burttey (24).

A pye unicam model 104 gas-liquid chromatography equipped with a flame ionization detector and a coiled glass column (1.5 m x 4 mm) packed with 10% polyethylene glycolic acid (PEGA) supported on acid w ashed diatomite C (100 120 mesh) was used. The sample (0.4 1.0 ul) was injected into the column using Hamilton microsyringe. The gas chromatographic condition used for temperature 170 °C, detector temperature 200 °C, range x 100, attenuator 5 x 10, hydrogen flow rate 45 ml/min., nitrogen flow rate 45 ml/min. and air flow rate 330 ml/min. The relationship between retention time and logarithm of carbon atoms number of fatty acids was made to take a knowledge about some unknown fatty acids. This relationship was drawn for even and odd numbers of fatty acids, as well as saturated and unsaturated fatty acids containing one, two or three double bonds, individually. Methyl ester of stearic acid was used as an internal standard to calculate relative retention time (RRT).

RESULTS

From previous studies, some researchers obtained fats which contained less than 0.25 µg of tocopherol/g fat (21). In this investigation, tocopherol was determined in cotton seed and corn oils using a HPLC method. The results given in table (1) show that the tocopherol contents in vitamin E-stripped oils were less than 20 µg/g oil.

Table 1. Tocopherol content of oils.

Oil		Tocopherol (µg/100g)	
	Corn oil	920	93
Vitamin	E-stripped corn oil	20	0.0
	Cotton seed oil	845	77
Vitamin	E-stripped cotton seed oil	20	0.0

Each value is a mean of 6

Tables (2,3) show the total fatty acids composition in hepatic tissues of rats fed on corn or cotton seed oils, respectively as a sole source of oil. All the trace amounts of fatty acids were considered in our calculations. The chromatograms illustrated the presence of 28 fatty acids, twenty four of which were identified in corn oil groups (Table 2) and twenty six of which were identified in cotton seed oil groups (Table 3). The remainders of fatty acids were unknown due to the unavailability of the authentic standards.

The previous data show that the total concentration of oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids in the liver of the control corn oil group were higher (22.9%) than that of the control cotton seed oil group (16.5%). However, hepatic linoleic acid in rats fed on corn oil was higher (19.2%) than that in rats fed on cotton seed oil (6.7%), but hepatic oleic acid in cotton seed oil group was higher (7.9%) than that of corn oil group (3.7%). Capric ($C_{10:0}$), lauric ($C_{12:0}$) and myristic ($C_{14:O}$) acids were also present in small quantities in livers of both groups. Fatty acids containing odd number of carbon atoms like $C_{11:0}$, $C_{13:0}$ and $C_{17:0}$ were also present in livers of rats of both groups.

A great decrease in linoleic acid in vitamin E-deficient corn oil group was found while it was completely absent in vitamin E-deficient cotton seed oil group, which was accompanied by an increase of palmitic ($C_{16:0}$), stearic ($C_{18:O}$) and oleic acids. Palmitic acid was also decreased in vitamin E-deficient corn oil group, while most of the short fatty acids (from C_6 to C_{12}) showed steadily increase.

Supplementation of vitamin E-stripped corn oil diet with excess of vitamin A increased the short chain fatty acids, some of these acids were not present in the control group such as $C_{6:0}$ (1.3%), $C_{6:1}$ (1.1%), $C_{10:1}$(2.7%) and $C_{11:1}$ (3.8%). In addition, this treatment produced five unknown short chain fatty acids, $C_{7:0}$, $C_{8:0}$, $C_{8:1}$, $C_{8:2}$ and $C_{9:0}$ were also increased. Livers of rats fed on a diet containing corn oil without vitamin E and with excess of vitamin A, contained 16.4% lauric acid, while the control group

and vitamin E-deficient group contained 7.3% and 8.1%, respectively. Linoleic acid content constituted about 19.2% of total fatty acids in livers of control rats fed on oil, while this value was decreased to 5.8% in vitamin E-deficient rats and highly decreased to 0.4% in vitamin E-deficient rats supplemented with vitamin A. Stearic, palmitic and myristic acids were also decreased, for example palmitic acid was decreased, from 28.3% to 7.9%.

Saturated and unsaturated short chain fatty acid (up to $C_{12:0}$) content of livers of fresh cotton seed oil group was not different from that of livers of vitamin E-stripped cotton seed oil group without or with excess of vitamin A. This result differed from that obtained by a vitamin A supplemented group which was fed on vitamin E-stripped corn oil. As shown in table (3), hepatic linoleic acid in cotton seed oil group was 6.7%, it was completely absent in vitamin E-stripped cotton seed oil group and it was slightly present (1.7%) by addition of vitamin E, but the content of oleic acid was increased from 9.8% in cotton seed oil group to 15.1% in vitamin E-stripped cotton seed oil group and to 13.3% in vitamin E-stripped cotton seed oil with excess of vitamin A. The total amount of stearic and palmitic acids together (the main saturated acids) was increased from 36.5% in cotton seed oil group to 53.7% after elimination of vitamin E and returned to 35.1% after addition of vitamin A.

Administration of vitamin C to vitamin E-stripped rats fed on corn oil free from vitamin E highly increased both hepatic stearic acid from 5.1% to 46.6% and oleic acid from 3.7% to 11.1%, respectively. Therefore, the percentages of most fatty acids were decreased, for example palmitic and linoleic acids -ere decreased from 28.3% to 8.7% and from 19.2% to 8.2%, respectively.

Traces of $C_{10:1}$ (15%) and $C_{11:1}$ (2.0%) were detected in the chromatogram of gas liquid chromatography, when rats fed on vitamin E-deficient cotton seed oil were given excess of vitamin C. Total saturated and unsaturated short chain fatty acids were increased from 26.3% to 43.2% by the same treatment. In addition, two unknown short chain fatty acids were detected the first constituted 1% and the second constituted 1.5% of the total fatty acids. The increase in short chain fatty acids was associated with the decrease in palmitic acid (from 24.2% to 10.8%), stearic acid (from 12.3% to 6.7%) and linoleic acid (from 6.7% to 3.9%). Oleic was increased from 9.7% to 13.1 %.

Small peaks of $C_{6:0}$ (3.0%) and two unknown fatty acids (1.9% and 0.3%) were detected in the chromatogram of hepatic fatty acids of rats fed on both vitamin E-

stripped corn oil and sodium selenite, as shown in table (2). The total content of fatty acids from $C_{6:0}$ to $C_{8:0}$ was 12.9% compared to 1.0% in fresh corn oil group. Short chain fatty acid content, in the corn oil group, constituted about 18.7% and the saturated: unsaturated ratio was 3:1 (13.9%: 4.4%). The former value was increased to 33.5%, but the ratio was not changed 3:1 (24.2 % : 7.1 %). This increase in short chain fatty acids especially from $C_{6:0}$ to $C_{8:0}$ was associated with the decrease in the linoleic acid content from 19.2% to 10.1%. In addition, oleic acid content was increased from 3.7% to 19.1%, and this increase was correlated with the decrease in the saturated fatty acids such as palmitic acid (from 28.3% to 13.3%) and meristic acid (from 4.2% to 1.9%)

Administration of sodium selenite by rats fed on vitamin E-stripped cotton seed oil slightly increased short chain fatty acids from 26.3% to 32.9%. This increase involved both short chain saturated fatty acids (from 19.7% to 23.9%) and unsaturated fatty acids (from 6.2% to 8.5%). Palmitic acid was decreased from 24.2% to 19.7% and fatty acids containing eighteen carbon atoms constituted about 22.0% compared to 28.8% in a cotton seed group (control group). This decrease involved stearic and linoleic acids.

DISCUSSION

The dietary oils used herein were specially prepared and do not simulate any natural or commercial product. Within the limits to practically available materials, the used cotton seed and corn oils were tocopherol stripped. These oils contained enough linoleic acid to meet the human requirements of essential fatty acids (25).

Our results (Table 2 and 3) are in agreement with data of Clement and Bourre (26) who found that the linoleic acid content and the total contents of unsaturated fatty acids in livers of rats fed on corn oil (highly unsaturated fatty acids content) were higher than that found in rats fed on cotton seed oil.

The presence of odd number fatty acids in liver may be attributed to the fact that, at the end of the experiment, the animals were decapitated without any starvation time and the digested lipids and fatty acids from the diet (corn or cotton seed oils) are transported directly into the liver. Thus, the lipid composition of the liver contained some odd number fatty acids which are naturally present in oils in small quantities. The metabolism of odd number fatty acids usually takes more time than that of even number fatty acids (27), this may illustrate the presence of odd fatty acids in liver.

Table 2. Hepatic fatty acid composition (% of total fatty acids) of rats fed on corn oil (control), vitamin E-stripped corn oil without or with excess of vitamin A, C or selenite ion.

Fatty acids	Corn oil (control)		Vit. E-stripped Corn oil		Vit. E-stripped Corn oil With Vit. A		Vit. E-stripped Corn oil With Vit. C		Vit. E-stripped Corn oil With Selentie ion	
%	%		%		%		%		%	
$C_{6:0}$	-		0.1	0.01	1.3	0.11	0.1	0.01	3.0	0.3
Unknown	-		1.0	0.09	-		0.1	0.01	1.9	0.09
*$C_{6:1}$	-		-		1.1	0.15	-		-	
$C_{7:0}$	0.2	0.01	-		1.6	0.2†			1.7	0.08†
Unknown	-		-		1.1	0.1	-		-	
$C_{8:0}$	0.3	0.04	1.8	0.2†	2.3	0.28†	0.1	0.02	2.2	0.19†
*$C_{8:1}$	0.2	0.03	-		3.4	0.4†	0.3	0.02	3.4	0.33†
*$C_{8:2}$	0.3	0.03	-		1.7	0.19†	-		0.7	0.05†
$C_{9:0}$	0.6	0.04	-		3.7	0.3†	0.3	0.27	1.9	0.2†
Unknown	0.3	0.02	-		1.1	0.1†	-		0.3	0.05
$C_{10:0}$	1.3	0.1	3.9	0.4†	4.3	0.3†	0.9	0.1	4.5	0.5†
*$C_{10:1}$	-		-		2.7	0.18	-		-	
Unknown	-		-		1.8	0.2	-		-	
*$C_{10:3}$	0.2	0.02	2.3	0.3†	4.1	0.05†	0.6	0.07†	0.4	0.32
Unknown	-		-		1.5	0.14	-		-	
$C_{11:0}$	4.2	0.35	6.7	1.0†	6.0	0.7†	1.6	0.2†	5.5	0.6
*$C_{11:1}$	-		-		3.8	0.2	-		-	
*$C_{11:2}$	1.6	0.19	2.3	0.2†	1.9	0.2	0.8	0.1†	1.2	0.08
*$C_{11:3}$	-		1.0	0.1	-		0.4	0.03	0.5	0.03
$C_{12:0}$	7.3	0.5	8.1	1.2	16.4	1.5†	2.3	0.25†	5.4	0.4†
*$C_{12:1}$	0.5	0.03	1.6	0.2†	1.4	0.13†	0.8	0.09	0.5	0.04
*$C_{12:2}$	1.6	0.2	1.8	0.2	4.2	0.5†	0.6	0.05	0.4	0.02
$C_{13:0}$	8.4	1.1	7.5	0.9	4.7	0.56†	2.0	0.18†	4.6	0.15†
Unknown	-		-		3.9	0.2	-		-	
*$C_{13:1}$	0.8	0.07	4.2	0.5†	-		1.1	0.1	0.9	0.07
Unknown	0.3	0.02	2.0	0.24	-		0.4	0.01		
*$C_{13:2}$	2.5	0.1	4.5	0.3†	2.3	0.4	1.0	0.08†	1.5	0.09†
Unknown	0.7	0.09	2.3	0.3†	1.6	0.15†	1.0	0.09	-	
Unknown	2.2	0.15	2.2	0.3	1.7	0.18	0.3	0.001†	-	
$C_{14:0}$	4.2	0.3	4.7	0.55	1.3	0.1†	1.4	0.1†	1.9	0.12†
$C_{14:1}$	2.0	0.16	3.3	0.28†	0.7	0.01†	1.8	0.12	1.2	0.09†
*$C_{14:2}$	1.0	0.1	3.2	0.3	0.6	0.01	0.8	0.05	0.4	0.03†
Unknown	-		-		0.3	0.03	3.8	0.2	-	
$C_{15:0}$	2.2	0.2	2.5	0.18	0.6	0.05†	0.8	0.5†	0.2	0.01†
$C_{16:0}$	28.3	4.0	15.4	2.4†	7.9	0.8†	8.7	0.9†	13.3	1.5†
$C_{16:1}$	-		1.1	0.13	0.3	0.04	1.0	0.07	2.6	0.16
$C_{17:0}$	1.2	0.2	1.2	0.15	-		-		0.5	0.03†
$C_{18:0}$	5.1	0.45	3.8	0.4	3.0	0.2	46.6	3.3†	7.1	0.9
$C_{18:1}$	3.7	0.4	5.9	0.7	7.6	1.0†	11.1	1.2†	19.1	2.7†
$C_{18:2}$	19.2	2.9	5.8	0.7†	0.4	0.3†	8.2	0.9†	10.5	1.1†
$C_{20:0}$	-		-		-		0.9	0.1	-	

* These acids were identified by a relationship between logarithm of retention time and carbon atoms number.
These values are means of 5

† Significant at 0.05.

Table 3. Hepatic fatty acid composition (% of total fatty acids) of rats fed on cotton seed oil (control), vitamin E-stripped cotton seed oil without or with excess amounts of vitamin A, C or selenite ion.

Fatty acids	Corn oil (control)		Vit. E-stripped Corn oil		Vit. E-stripped Corn oil With Vit. A		Vit. E-stripped Corn oil With Vit. C		Vit. E-stripped Corn oil With Selentie ion	
%	%		%		%		%		%	
$C_{8:0}$	0.6	0.05	0.2	0.01†	0.6	0.04	1.4	0.08†	1.2	0.07†
* $C_{8:1}$	0.9	0.07	0.3	0.01†	1.4	0.09†	1.8	0.05†	1.9	0.06†
* $C_{8:2}$	0.4	0.04	0.2	0.02	0.3	0.01	1.2	0.07†	0.5	0.03
Unknown		-		-		-	1.0	0.04		-
$C_{9:0}$	1.3	0.02	0.5	0.02†	1.4	0.06	1.9	0.11†	2.0	0.1†
Unknown	0.4	0.03	0.2	0.01	0.4	0.02	1.4	0.12†	0.5	0.02
$C_{10:0}$	3.2	0.25	1.6	0.05†	3.4	0.2	4.1	0.25†	4.5	0.2†
* $C_{10:1}$		-		-		-	1.5	0.08		-
* $C_{10:2}$	0.3	0.02	0.3	0.01	0.2	0.01	1.7	0.03†	0.4	0.02
* $C_{10:3}$	0.6	0.05	0.4	0.01	0.4	0.02	2.2	0.09†	0.7	0.05
$C_{11:0}$	7.0	0.6	3.6	0.1†	6.4	0.07	5.4	0.2†	8.0	0.9
* $C_{11:1}$		-		-	0.4	0.03	2.0	0.1		-
* $C_{11:2}$	1.4	0.09	1.1	0.06	1.3	0.09	2.5	0.1†	1.6	0.08
* $C_{11:3}$	0.5	0.03	0.3	0.01	0.5	0.03	2.0	0.07†	0.6	0.03
$C_{12:0}$	7.6	0.5	5.0	0.2†	7.9	0.6	7.1	0.6	8.2	0.7
* $C_{12:1}$	0.4	0.02	0.5	0.03	0.6	0.04	1.9	0.09†	0.5	0.03
* $C_{12:2}$	1.7	0.08	1.7	0.11	1.9	0.05	4.1	0.2†	2.3	0.1†
$C_{13:0}$	7.0	0.4	4.6	0.3†	6.7	0.04	5.4	0.25†	7.3	0.5
* $C_{13:1}$	1.1	0.07	1.3	0.1	1.9	0.1†	2.0	0.06†	1.2	0.08
Unknown		-	0.4	0.02		-		-	0.9	0.04
* $C_{13:2}$	2.1	0.09	1.4	0.015†	2.3	0.1	3.2	0.1†	2.0	0.08
Unknown	1.8	0.1		-		-		-	1.8	0.1
$C_{14:0}$	2.8	0.1	2.1	0.1†	3.1	0.1	2.7	0.2	2.9	0.1
$C_{14:1}$	1.6	0.08		-	1.2	0.08	2.7	0.15†	1.3	0.1
* $C_{14:2}$	0.7	0.03	0.6	0.04	1.1	0.09	1.0	0.04	0.7	0.03
Unknown		-	2.9	0.15	1.7	0.05		-	2.0	0.09
$C_{15:0}$	1.4	0.05	1.2	0.07	1.5	0.09	0.7	0.05†	1.6	0.1
$C_{16:0}$	24.2	2.5	33.8	2.9†	21.7	1.9	10.8	0.7†	19.7	1.5
$C_{16:1}$	0.5	0.03	0.3	0.02	1.1	0.02†	0.9	0.05	0.9	0.07
$C_{17:0}$	0.9	0.04		-	0.9	0.02†	0.5	0.02	0.9	0.06
$C_{18:0}$	12.3	1.1	19.9	1.5†	13.4	1.0	6.7	0.4†	8.1	0.5†
$C_{18:1}$	9.8	0.8	15.1	1.2†	13.3	0.08	13.1	1.0	10.3	0.8
$C_{18:2}$	6.7	0.03		-	1.7	0.1†	3.9	0.2†	3.6	0.2†

* These acids were identified by a relationship between logarithm of retention time and carbon atoms number.
These values are means of 5

† Significant at 0.05.

Various investigations have studied the vitamin E-deficient rats at an early stage of deficiency (10) and found that the hepatic fat had a lesser concentration of linoleic acid than the control animals. Similar results were obtained on tables 2 and 3. The absence of hepatic linoleic acid in vitamin E-deficient rat may show the strong lipid peroxidation and degradation of polyunsaturated fatty acids (26).

Total short chain fatty acids (from C_6 to C_{12}) in the corn oil group and vitamin E-stripped corn oil group with or without excess of vitamin A was 18.6%, 65.4% and 30.6%, respectively. This means that vitamin E-deficiency, especially in the presence of large quantities of vitamin A, increased the autoxidation of long chain fatty acids, therefore, short chain fatty acids, were increased. The unsaturated short chain fatty acids in hepatic lipid constituted about 4.4% of total fatty acids in rats fed corn oil, this value was evaluated to be 9.0% when vitamin E was stripped from corn oil. The unexpectedly highest value of the unsaturated short chain fatty acids (24.3%) was obtained in the presence of high dose of vitamin A. It may be shown that vitamin A could not replace the antioxidant function of vitamin E, in the presence of corn oil as a sole source of lipids.

Linoleic acid content constituted about 19.2% (table 2) of total fatty acids in livers of control rats fed on corn oil, while this value was decreased to 5.3% in vitamin E-stripped corn oil and highly declined to 0.4% in vitamin E-stripped corn oil with excess of vitamin A. These results confirmed the previous results, in which unsaturated long chain fatty acid ($C_{18:2}$) was autoxidized to short saturated and unsaturated fatty acids in the absence of vitamin E with or without excess of vitamin A.

Administration of excess of vitamin A to vitamin E-stripped cotton seed oil group decreased linoleic acid content, but short chain fatty acids were not changed. In addition, oleic acid was increased. Therefore, these results may indicate that administration of vitamin A by vitamin E-deficient rats, which depend in there died upon vitamin E-stripped cotton seed oil is acceptable, while those rats fed vitamin E-stripped corn oil with excess of vitamin A, gave results which are completely unacceptable.

In vitamin E-stripped corn oil group with large amounts of vitamin C, the total content of fatty acids containing eighteen carbon atoms ($C_{18:0}$, $C_{18:1}$ and $C_{18:2}$) was increased to 65.9%. Stearic acid was also highly increased. These data indicate that vitamin C may encourage the formation of stearic acid.

Excess of vitamin C in vitamin E-stripped cotton seed oil group decreased the ratio between saturated and unsaturated long chain fatty acids (more than $C_{12:0}$) from

2:1 (48.6%: 22.5%) to 1:1 (26.8%: 26.8%). Vitamin C, which is a water soluble antioxidant, could decrease the lipid peroxidation especially in the presence of cotton seed oil in a diet.

Administration of adjustable amounts of sodium selenite by vitamin E-deficient rats fed on corn oil was very beneficial, since it increased stearic, oleic and linoleic acids from 3.8% to 7.1%, from 5.9% to 19.1% and from 5.8% to 10.5% respectively. These amount of acids may be more suitable for fatty acid composition in livers. Sodium selenite could improve the pattern of fatty acids containing eighteen carbon atoms.

In conclusion, in vitamin E-deficient corn oil groups administration of sodium selenite is more efficient than administration of vitamin A or C. This conclusion depends upon that the total amount of the stearic acid family ($C_{18:0}$, $C_{18:1}$ and $C_{18:2}$) was 36.7% and the linoleic acid constituted 10.5%. The relationship between stearic acid and linoleic acid was 0.68 : 1. These values were the nearest to the values obtained by force feeding of rats on commercial diets (23). Vitamin C followed sodium selenite and increased these acids to 65.9%, but linoleic acid content was decreased to 8.2% and stearic acid constituted 46.6%. In this case, the ratio between stearic and linoleic acid was very high (5.7:1). Vitamin A should not be used instead of vitamin E as an antioxidant, since the total content of stearic acid family did not exceed than 11% and the linoleic acid content was dramatically decreased (0.4%).

In the case of vitamin E-deficient rats fed on cotton seed oil free from vitamin E, as a sole source of oil, administration of vitamin C or sodium selenite gave similar results, since the amounts of stearic acid (6.7% and 8.1%) and oleic acid (13.1% and 10.1%) were nearly similar to those found in normal rats, while, the quantity of linoleic acid (3.9% and 3.6%) was different from that found in rats fed on commercial diets. Therefore, the ratio between stearic acid and linoleic acid varied between 1.7 and 2.3, respectively. This ratio was highly increased (7.8) by administration of vitamin A. In the present study, the total contents of stearic, oleic and linoleic acids after administration of vitamin C, selenite or vitamin A were 23.7%, 12.9% or 28.4%, respectively.

REFERENCES

1. Kretzer, F.L., Hittner, H.M., Johnson, A.T., Mehta, R.S. and Godio, L.B. (1982) : Vitamin E and retrolental fibroplaisa ultrastructural support of clinical efficiency. Ann. NYA Cad Sci. 393: 145 166.

2. Craig, E.T. and Reed, D.J. (1990): Radical induced inactivation of kidney Na^+, K^+ ATPase: Sensitivity to membrane lipid peroxidation and the protective effect of vitamin E. Arch. Biochem. Biophys., 281 : 96 105.

3. Dewys, E.D., Malone, W.F., Butrum, R.R., and Sestili, M.A. (1986): Clinical trials in cancer prevention. Cancer 58 (suppl 8), 1962 1964.

4. Chow, C.K. (1990): Effect of dietary vitamin E and selenium on rats; pyruvate Kinase, glutathione peroxidase and oxidative damage. Nutr. Res., 10, 183 194.

5. Curphey, T.J., Kuhlmann, E.T., Roebuck, B.D., and Longenecker, D.S. (1988): Inhibition of pancreatic liver carcinogenesis in rats by retino and selenium supplemented diets. Pancreas 3: 36 40.

6. Clement, M. and Bourre, J.M. (1993): Alteration of brain and liver microsomal polyunsaturated fatty acids following dietary vitamin E-deficiency. Neur. Sci. Letters, 164, 163 166.

7. Willson, R.P., Bowsr, P.R. and Poe, W.E. (1983): Dietary pantothenic and requirement of fingerling channel catfish. J. Nutr. 113, 2124 2128.

8. Despert, S., Dinh, L., Clement, M. and Bourre, J.M. (1992): Alteration of delta-desaturase by vitamin E in rats brain and liver. Neuro Sci. Lett., 145, 19 22.

9. Willson, R.P. (1977): Free radials and electron transfer in biology and medicine. Chem. Ind.: 183 - 193

10. Dogterm, P. and Mulder, G. L. (1993) : Different effects of extracellular calcium on lipid peroxidation dependent (Ethacrynic acid Allyl Alcohol) and lipid peroxidation independent (Disulfiram) induced cytotoxicity in normal and vitamin E-deficiency Rat hepatocytes. Chem. Biol. Interactions, 86: 171 181.

11. Tappel, A.L. (1968): Will antioxidant nutrients show processes Geriatrics 23 : 97 105.

12. Packer, J.E., Mahood, J.S., Mosra-Arellano Vo, Slater, T.F., Willson, R.L. and Wolfenden, B.S. (1981): Free radicals and singlet oxygen Scavengars: reaction of aperoxy radical with B-Carotene, diphenylfuran and 1.4 diazabicyclo (2,2,2) Octane. Biochem. Biophys. Res. Commun 98: 901 906.

13. Frankel, E.N. (1989): The antioxidant and nutritional effects of Tocopherols, ascorbic acid and Beta-carotene in relation to process of edible oils. Bibl. Nutr. Dieta, 43: 297 312.

14. Burton, G.W., Wronska, U., Stone, L., Foster, D.O. and Ingold, K U. (1990): Biokinetics of dietary RRR- ∝ - Tocopherol in the male Guinea Pig at three levels of vitamin C and t\N o levels of vitamin E. Evidence that vitamin C does not 25, 4, 199 210.

15. Dam, H. (1962) : Interrelations between vitamin E and polyunsaturated fatty acids in animals. Vitamins and Hormones, 30:527.

16. Horwitt, M.K. (1962): Vitamin E and lipid metabolism in man. Am. J. Clin. Nutr., 8: 451 461.

17. Harris, P.L. and Embree, N.D. (1963): Quantitative consideration of the effect of polyunsaturated fatty acid content of the diet upon the requirements for vitamin E. Am. J. Clin. Nutr., 13: 385.

18. Bieri, J.G., and Evarts, R.P. (1973): Tocopherol and fatty acid in American diet. The recommended allowance for vitamin E. J. Am. Diet. Asoc. 62: 147 151.

19. Harmon, E.M., Witting, L.A. and Horwitt, M.K. (1991): Relative rates of depletion of ∝ - tocopherol and linoleic acid after feeding polyunsaturated fats.

20. Lomnitski, L., Bergman, M., Schon, I. and Grossman, S. (1991): The effect of dietary vitamin E and beta-carotene on oxidation process in the rat testes. Lipids and lipid Metabolism. 1082, 1: 101 107.

21. Witting, L.A. and Horwitt, M.K. (1964) : Effect of degree of fatty acid unsaturated in tocopherol deficiency induced creatinuria. J. Nutr. 82: 19.

22. Vatassery, G.T. and Smith, W.E. (1987) : Determination of ∝-tocopherol quinone (Vitamin E Quinone) in Human serum, platelets, and red cell Membrane Samples. Analytical Biochemistry 167: 411 417.

23. A.O.A.C. (1975) : Official methods of analysis. 12th. ed., Associated of Official Agricultural Chemists. Washington. D.C.

24. Getz, .J. R. and Burttey, G.E.R. (1961): The intracellular distribution of fatty acids in rat liver. The fatty acids of intracellular compartments. Biochem. J. 78 :307.

25. Mohrover, S.L. and Holman, A.C. (1969): Effect of dietary antioxidants and phenobarlbital pretreatment on microsomal lipid peroxidation and activation by carbon tetrachloride. Life Sci. 19: 1 1 5 1 1 1 60.

26. Clement, M. and Bourre, J.M. (1990): Alteration of ∝ - tocopherol content in developing and aging peripheral nervous system. Persistence of high correlations with total and specific (n-6) polyunsaturated fatty acids. J. Neurochem., 54 : 2110 2117.

27. Vogel, H.G. (1971): Metabolic Pathways. Third edition Vol. 5. New York, Academic press, P. 23.

تأثير بعض مضادات الاكسدة على مستوى الاحماض الدهنية فى كبد الفئران التى تعانى من نقص فيتامين E

صائب عبد المنعم حافظ [١]، سمير عبد المنعم اسماعيل [٢]

١ قسم *الاغذية الخاصة – معهد بحوث تكنولوجيا الاغذية – مركز البحوث الزراعية*
٢ قسم *الكيمياء الحيوية – كلية الزراعة – جامعة القاهرة*

يهدف البحث الى ايجاد اجابة محددة عن هل من الممكن ان يحل فيتامين A او C او ايون السلينيت محل الفعل المضاد للاكسدة لفيامين E متخذين الاحماض الدهنية فى الكبد كمقياس لذلك. وفى هذا البحث غذيت الفئران على عليقة قياسية تحتوى على زيت الذرة او زيت القطن المزال منها فيتامين E لمدة ٤٢ يوم. كميات اضافية من أى من فيتامين A (٠,٤٥ جرام/يوم) او فيتامين C (٧,٥ ملليجرام/يوم) او سيلينيت الصوديوم (٠,١ ملليجرام/يوم) او الماء اعطيت يوميا عن طريق الفم بواسطة انبوبة خاصة. اجريت تجربة مقارنة على فئران تتغذى على عليقة تحتوى على زيت الذرة أو زيت القطن كمصدر للدهون لمدة ٦ اسابيع. وتشير النتائج الى أن هناك انخفاض كبير فى محتوى اللينوليك فى المجموعة التى كانت تتغذى على زيت الذرة الخالى من فيتامين E واختفى تماماً من المجموعة التى تتغذى على زيت القطن الخالى من فيتامين E. وقد وجد أن إضافة فيتامين A فى وجود زيت القطن الخالى من فيتامين E اعطى نتائج طيبة بينما فى حالة وجود هذا الفيتامين مع زيت الذرة الخالى من فيتامين E وجد أنه حدث اكسدة ذاتية لحمض اللينوليك وظهور احماض دهنية قصيرة السلسلة بكميات كبيرة نسبياً. وقد وجد ان وجود فيتامين C مع زيت الذرة الخالى من فيتامين E احدث زيادة كبيرة فى حمض الاستياريك من ٥,١ ٪ الى ٤٦,٦ ٪ وكذلك حمض الاوليك من ٣,٧ ٪ الى ١١,١ ٪. اما إضافة ايون السيلينيت الى مجموعة زيت الذرة الخالى من فيتامين E احدث زيادة طفيفة فى الاستيارك والاوليك واللينوليك. والنتائج تشير الى ان ايون السلنيت كان احسن المواد المضادة للاكسدة فى حالة زيت الذرة المنزوع منه فيتامين E كما انه عمل على تحسين مستوى الاستيارك والاوليك واللينوليك. اما فى حالة مجموعة زيت القطن الخالى من فيتامين E فان اضافة كميات كبيرة من سيلينيت الصوديوم او فيتامين C اعطيت نتائج متشابهة ومقبولة.