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Liver and Plasma Lipids in Vitamin E-Deficient Rats

MASAHIDE YASUDA, TADASHI FUJITA, and YASUHISA MIZUNOYA

Osaka College of Pharmacy, Department of Hygienic Chemistry[1)]

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To investigate metabolic alterations during vitamin E-deficiency, the content of lipid peroxides and lipids in liver and plasma was determined using vitamin E-deficient rats, and its results were as follows: Lipid peroxide in the liver from vitamin E-deficient rats increased about 3 times over the control group, and the value returned to the normal level after intraperitoneal administration of vitamin E in a dose of 50 mg/kg/day for 6 days, but that in plasma was almost constant. In vitamin E-deficiency, $C_{16:1}$, $C_{18:1}$, and $C_{20:4}$ increased mainly in triglycerides (TG) in liver lipids. $C_{18:2}$ decreased in the fractions of phospholipids but not in TG fraction. On the other hand, in plasma from vitamin E-deficient rats, $C_{18:1}$ increased and $C_{18:2}$ decreased in all the fractions of lipids. Decrease of $C_{20:4}$ was found in phosphatidylethanolamine (PE) and phosphatidylcholine (PC) in plasma lipids. Free fatty acids of plasma in vitamin E-deficiency increased by approx. 76%, and TG content increased about 73% in the liver and 35% in the plasma. The total cholesterol content of the liver and plasma from vitamin E-deficient rats increased about 55 and 20%, respectively. The content of phospholipids in the liver and plasma showed little change in vitamin E-deficiency.

Keywords——lipids; lipid peroxide; plasma; liver; vitamin E-deficiency

The biological action of vitamin E has been studied for many years since this vitamin was first shown by Evans and Bishop as the antisterility activity.[2)] However, there is little unequivocal evidence that vitamin E is of nutritional significance or is of any value in therapy.

Lucy[3)] and Green[4)] have shown that the main function of vitamin E in biological systems is an antioxidant, and that the molecules protected from oxidation are polyunsaturated fatty acids in phospholipids of subcellular membranes.

Therefore, to investigate metabolic alterations during vitamin E-deficiency, content of lipids in the liver and plasma was determined using vitamin E-deficient rats.

Experimental

Treatment of Animals and Drugs——Wistar strain male rats, weighing 150—250 g, were used for the experiments. Normal rats were fed a vitamin E-deficient diet (Oriental Yeast Co., Tokyo), and the control animals were fed a standard laboratory diet (Oriental Yeast Co., Tokyo) supplemented with 100 mg of α-tocopheryl acetate per kg of food for 3 weeks. Vitamin E (DL-α-tocopherol) was suspended in an appropriate amount of 0.1% Tween 80, and 50 mg/kg was given to vitamin E-deficient rats intraperitoneally once daily for 6 consecutive days. Vitamin E-deficiency state was determined by the hemolysis test of Ikehata and Sugiyama.[5)] Hemolysis percentages in the vitamin E-deficient and in the normal groups were about 70% and 12%, respectively.

Preparation of Liver and Plasma——The animals were sacrificed by decapitation, blood samples were collected into a heparinized centrifuge tube, and then blood was centrifuged at 3000 rpm for 5 min at room temperature. The liver removed rapidly from the decapitated rat was washed with ice-cold saline solution, and 10% (w/v) homogenate was prepared with 0.15 M KCl–0.02 M Tris-HCl buffer (pH 7.4), using a Potter-Elvehjem Teflon homogenizer. An aliquot of both plasma and liver homogenates was used to determine the content of lipids and lipid peroxides.

1) Location: *2–10–65, Kawai, Matsubara, Osaka, 580, Japan.*
2) H.M. Evans and K.S. Bishop, *Science*, **56**, 650 (1922).
3) J.A. Lucy, *Ann. N.Y. Acad. Sci.*, **203**, 4 (1972).
4) J. Green, *Ann. N.Y. Acad. Sci.*, **203**, 29 (1972).
5) H. Ikehata and Y. Sugiyama, *Vitamins*, 37, 31 (1968) (in Japanese).

Assay Methods of Fatty Acid Composition——Phospholipids and triglycerides in the liver and plasma were separated by thin-layer chromatography (TLC). The plate (Silica Gel H, from Merck) was developed in the usual way with a mixture of chloroform–methanol–acetic acid–water (85: 15: 10: 4, v/v) for phospholipids, and with petroleum ether–ether–acetic acid (80: 30: 1, v/v) for triglycerides. The spots were visualized by spraying the plate with 1% iodine in methanol. After evaporation of iodine, spots of phospholipids and triglycerides were scraped off for fatty acid analysis, and the fatty acid composition in these fractions of liver and plasma was determined by gas liquid chromatography (Shimadzu Chromatograph, Model GC-1C, GLC) with hydrogen flame ionization detector.[6] The column packed with 15% ethyleneglycol-adipate polyester on Chromosorb WAW (60—80 mesh) was operated at 200° with a nitrogen flow rate of 60 ml/min. The methyl esters of fatty acids were obtained by methylation with 5% HCl–methanol for 2 hr under reflux, and were identified by comparison with the retention time of authentic standards.

Determination of Lipids and Lipid Peroxide——Content of triglycerides in the liver and plasma was assayed colorimetrically by the procedure of Naito *et al.*,[7] and that of free fatty acids by the method of Itaya and Ui.[8] Total cholesterol and phospholipids were measured by the method of Abell *et al.*[9] and of Bartlett,[10] respectively. Content of lipid peroxides in the liver and plasma was estimated by the thiobarbituric acid (TBA) reaction as described by Tappel and Zalkin,[11] and it was expressed as TBA values (absorbance at 532 nm/mg tissue (ml), for liver and plasma).

Statistical Evaluation——The number of animals used and significance of the results, evaluated by means of Student's t-test, are shown in the tables.

Results and Discussion

Effect of vitamin E-deficient on lipid peroxidation in the liver and plasma is shown in Table I. Content of lipid peroxides is expressed as TBA values (absorbance at 532 nm/mg tissue (ml), for liver and plasma). Lipid peroxides in the liver from vitamin E-deficient rats increased about 200% ($p<0.01$), compared to that of control animals, and in addition the value returned to the control level after intraperitoneal administration of vitamin E to vitamin E-deficient rat, in a dose of 50 mg/kg/day for 6 days. However, no significant difference was observed in the plasma lipid peroxide contents between normal rats and vitamin E-deficient groups. It is well known that polyunsaturated fatty acids in phospholipids of membranes are utilized as the substrate of lipid peroxidation, so that the fractions of triglycerides and phospholipids in liver and plasma were separated by TLC, and the fatty acid composition was determined by GLC. As shown in Table II, monoenoic acids in the fraction of TG increased in vitamin E-deficiency, and $C_{18:2}$ in PE and PC decreased markedly, although no appreciable change was found in $C_{18:2}$ of TG fraction. It has been reported that

Table I. Content of Lipid Peroxides in Rat Liver and Plasma

Treatment	TBA-value	
	Liver OD at 532 nm/mg tissue	Plasma OD at 532 nm/ml
	Mean±S.E. ($n=4$)	Mean±S.E. ($n=4$)
Control	0.053±0.001	0.293±0.011
VE-deficiency	0.171±0.003[b]	0.332±0.039
VE-deficiency+VE[a]	0.056±0.002	0.309±0.017

a) *i.p.* administration of DL-α-tocopherol (50 mg/kg/day, ×6).
b) significantly different from the control group ($p<0.01$).

6) W. Stoffel, F. Chu, and E.H. Ahrens, *Anal. Chem.*, **31**, 307 (1959).
7) C. Naito, M. Usui, K. Kohayakawa, H. Okaniwa, and T. Ichida, *Igakunoayumi*, **57**, 551 (1966) (in Japanese).
8) K. Itaya and M. Ui, *J. Lipid Res.*, **6**, 16 (1965).
9) L.L. Abell, B.B. Levy, B.B. Brodie, and F.E. Kendall, *J. Biol. Chem.*, **159**, 357 (1952).
10) G.R. Bartlett, *J. Biol. Chem.*, **234**, 466 (1959).
11) A.L. Tappel and H. Zalkin, *Arch. Biochem. Biophys.*, **80**, 326 (1959).

arachidonic acid content of liver lipids increased in vitamin E-deficient state,[12,13] but the mechanism is not yet clear. As was seen with TG fraction, $C_{20:4}$ increased about four-fold in vitamin E-deficiency, but $C_{20:4}$ showed little change in fractions of phospholipids. On the other hand, $C_{18:1}$ increased and $C_{18:2}$ decreased in all the fractions of plasma lipids in vitamin E-deficiency (Table III).

Table II. Fatty Acid Composition of Different Lipid Fractions in Liver from Vitamin E-Deficient Rats[a]

Chain length	Treatment	TG	PE	PC
$C_{16:0}$	Normal	43.31	24.59	25.13
	VE-def.	21.96	26.70	35.76
$C_{16:1}$	Normal	+	+	+
	VE-def.	7.11	+	+
$C_{18:0}$	Normal	21.71	25.81	29.43
	VE-def.	6.21	37.39	31.21
$C_{18:1}$	Normal	16.23	5.22	6.39
	VE-def.	39.91	3.28	8.78
$C_{18:2}$	Normal	15.82	13.88	21.47
	VE-def.	14.35	2.09	3.93
$C_{20:4}$	Normal	2.93	23.08	17.58
	VE-def.	12.53	26.52	20.32

Only the major acids are listed. Plus signs indicate trace.
a) Duplicate analyses of pooled liver from 5 animals.
Abbreviations are TG; triglyceride, PE: phosphatidylethanolamine, and PC; phosphatidylcholine.

Table III. Fatty Acid Composition of Different Lipid Fractions in Plasma from Vitamin E-Deficient Rats[a]

Chain length	Treatment	TG	PE	PC
$C_{16:0}$	Normal	32.09	50.29	31.46
	VE-def.	32.10	56.02	32.77
$C_{16:1}$	Normal	+	+	+
	VE-def.	5.05	+	+
$C_{18:0}$	Normal	3.71	21.42	27.26
	VE-def.	9.22	32.21	30.09
$C_{18:1}$	Normal	33.84	5.22	5.38
	VE-def.	47.63	9.70	26.91
$C_{18:2}$	Normal	30.34	5.78	24.31
	VE-def.	5.12	+	6.54
$C_{20:4}$	Normal	+	17.27	11.59
	VE-def.	+	+	1.00

Only the major acids are listed. Plus signs indicate trace.
a) Duplicate analyses of pooled plasma from 7 animals.
Abbreviations are TG; triglyceride, PE; phosphatidylethanolamine, and PC; phosphatidylcholine.

12) K. Bernhard, S. Leisinger, and W. Perderson, *Helv. Chim. Acta*, **46**, 1767 (1963).
13) E.M. Harmon, L.A. Witting, and M.K. Horwitt, *Am. J. Clin. Nutr.*, **18**, 243 (1968).

Table IV. Content of Lipids in Rat Liver and Plasma

Treatment	Liver	Plasma
	Free fatty acids (μEq/g tiseue)	(mEq/l)
Control	3.13 ±0.32 (n=20)	0.406±0.024 (n=20)
VE-deficiency	3.87 ±0.19 (n=20)	0.716±0.032[b] (n=20)
VE-deficiency+VE[a]	3.99 ±0.31 (n=20)	0.594±0.037[b] (n=20)
	Triglycerides (mg/g tissue)	(mg/dl)
Control	5.768±0.026 (n=12)	63.06 ±4.01 (n=12)
VE-deficiency	9.962±0.725[b] (n=12)	85.27 ±7.60[c] (n=12)
VE-deficiency+VE[a]	8.963±0.340[b] (n=12)	87.26 ±2.06[b] (n=12)
	Total cholesterol (mg/g tiseue)	(mg/dl)
Control	2.27 ±0.13 (n=20)	47.27 ±1.45 (n=20)
VE-deficiency	3.52 ±0.21[b] (n=20)	56.67 ±2.02[c] (n=20)
VE-deficiency+VE[a]	2.90 ±0.16[c] (n=20)	58.30 ±1.52[b] (n=20)
	Phospholipids (Pi mg/g tissue)	(Pi mg/dl)
Control	1.338±0.045 (n=20)	2.862±0.082 (n=20)
VE-deficiency	1.345±0.030 (n=14)	2.929±0.064 (n=20)
VE-deficiency+VE[a]	1.236±0.026 (n=14)	2.686±0.153 (n= 8)

a) *i.p.* administration of DL-α-tocopherol (50 mg/kg/day, ×6).
b) significantly different from the control group ($p<0.01$).
c) significantly different from the control group ($p<0.05$).
Values are the mean±S.E.

Table IV shows the content of lipids in the liver and plasma. In vitamin E-deficiency, plasma free fatty acids increased by approx. 76% ($p<0.01$), and this value was little affected even though vitamin E was given for 6 days, although the TBA value was returned to the normal level. This may imply that the effect is due to lowering in transport of the fatty acids from the plasma into liver or peripheral tissues. TG in vitamin E-deficiency increased about 73% in the liver and about 35% in the plasma, and these values were still high after vitamin E was given for 6 days, and cholesterol content also increased in the both. The phospholipid content in the liver and plasma showed no change in vitamin E-deficiency.

It has been demonstrated that a multiplicity of deficiency manifestations occur in different animals and in various organs when the animals are fed on a vitamin E-deficient diet, and the manifestations can be recovered by the administration of vitamin E.[14] Recently, Hulstaert and Molenaar[15] reported that vitamin E-deficiency state leads to changes in the activity of a number of membrane-bound enzymes and alterations in a part of protein in the intracellular membranes, and a deficiency of this vitamin has an effect not only on lipids but also on protein of membranes.

It is generally accepted that vitamin E as an antioxidant is particularly important as a scavenger of free radicals which can be induced, at least partly, by polyunsaturated fatty acids in phospholipids of membranes.[16] In the present experiment, the content of lipid peroxides in liver was markedly increased by the vitamin E-deficiency state, and $C_{18:2}$ in phospholipids of the liver decreased. In such a case, the lipid peroxides returned to the normal level after treatment of vitamin E-deficient rats with vitamin E. Therefore, it may be conceivable

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15) C.E. Hulstaert and I. Molenaar, *Int. J. Vitam. Nutr. Res.*, **46**, 262 (1976).
16) P.B. McCay, P.M. Pfeifer, and W.H. Stipe, *Ann. N.Y. Acad. Sci.*, **203**, 62 (1972).

that the vitamin acts as a scavenger of free radicals from polyunsaturated fatty acids in biological system.

In vitamin E-deficient rats, a clear percentage increase in monoenoic acids was observed in lipids of the liver and plasma. The observed increase of monoenoic acids, particularly, $C_{18:1}$, reflects, at least in part, to the fraction in the liver TG and all the fractions of lipids in the plasma. On the other hand, decrease of $C_{18:2}$ may be induced from the fractions of phospholipids in the liver, and from all the fractions of lipids in the plasma.