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Lipid Peroxidation and Vitamins E and A Levels in Tissues of Rats Fed Fish Oil or Soybean Oil Supplemented with Vitamin E*

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ABSTRACT

To investigate effects of dietary fish oil and vitamin E level on the tissue levels of vitamin E and vitamin A and to see which tissue is sensitive to lipid peroxidizability, male Sprague-Dawley rats were fed experimental diets composed of either menhaden oil or soybean oil and either low(equivalent to 17 mg α-tocopherol) or high (equivalent to 140 mg α-tocopherol) vitamin E level for 4 weeks. Plasma TBARS per mg lipid was significantly elevated in rats fed fish oil with low vitamin E level compared to soybean oil-fed rats. TBARS levels of liver, heart, kidney and liver microsomes were also increased by feeding fish oil with low vitamin E level. Plasma TBARS level was significantly correlated with TBARS levels of liver, heart, kidney and liver microsomes. Plasma vitamin E level of groups with vitamin E supplementation was elevated significantly as compared to those without vitamin E supplementation, whereas vitamin E levels of liver, heart and kidney were not changed significantly. Plasma TBARS was negatively correlated with plasma vitamin E($r=-0.5763$, $P<0.001$) and A($r=-0.4523$, $P<0.01$) and seems to be a good indicator of in vivo lipid peroxidative stress.

KEY WORDS : lipid peroxidation · TBARS · vitamin E · vitamin A · fish oil.

Introduction

During past two decades, beneficial effects of marine oils on prevention and retardation of coronary heart disease and other illnesses have been recognized by epidemiological data. Underlying mechanisms for the effects have been extensively studied by numerous animal experiments and human trials[1] However, due to its high unsaturation, fish oil can be rapidly oxidized to produce lipid peroxide. Several studies have shown increases in serum or plasma and tissue contents of thiobarbituric acid-reactive substances(TBARS)

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in animals[2-5] and humans[6-8] fed fish oil or fish oil concentrates. Since cellular enrichment of long-chain n-3 fatty acids of high peroxidizability is likely to render tissues more susceptible to oxidation, the requirement for antioxidant nutrients would increase[5)8)].

In a previous work[10], some effects of dietary lipids and vitamin E on lipid peroxidation were tissue-specific, and, though lipid peroxide level of liver increased dramatically by fish oil as compard to soybean oil and decreased by increasing the level of dietary vitamin E, the liver vitamin E level was influenced by dietary fat at much lower extent and the vitamin A level was without effect. A similar finding was reported by Hu et al[4]. Leibovitz et al[5] have also reported that, while lipid peroxide level of rats fed menhaden oil was much higher than that of corn oil-fed rats, the source of lipid was without effect on tissue vitamin E. These results were out of accordance with a result reported by Chautan et al[9]. They have observed that the liver α-tocopherol level dropped dramatically when n-3 polyunsaturated fatty acids were gradually added to the diet for rats.

Although Vitamin E is an excellent lipid-soluble, chain-breaking antioxidant, but it can act as a pro-oxidant. Very little is known about the determinants of the levels and turnover of vitamin E in individual tissues [11], and changes in the status of other antioxidants do occur following viamin E consumption[10]. According to the work of Napoli et al[12], vitamin A metabolism was affected by tissue α-tocopherol concentration in several tissues, and the effect was tissue-specific.

Therefore, the present study was undertaken to investigate whether lipid peroxidation was affected by the peroxidative susceptibility of dietary lipid and vitamin E level, whether tissue levels of vitamin E and A could be altered by vitamin E supplementation, and which tissue would be the most sensitive by the increased lipid peroxidation and could be used as an indicator for oxidative stress in vivo.

Materials and Methods

Diets and animals

Four groups of 8-10 male Sprague-Dawley rats (Experimental Animal Management Division, National Institute of Health, Seoul, Korea) weighing about 150g were housed individually and were fed a vitamin E-deficient diet, of which the composition was the same as that of the experimental diets(Table 1) except that lard oil(Sigma Chemical Co., St. Louis, MO, U.S.A.) was added instead of soybean oil or menhaden oil and vitamin E was not supplemented, for 2 weeks. Then each group was fed one of the experimental diets containing either 10%(w/w) menhaden oil or 10% soybean oil(Haepyo Co., Korea) with two levels of viatmin E for each of the two oils(Table 1) for 4 weeks. The levels of vitamin E, 17 α-TE and 140 α-TE, correspond to a half and 4 times, respectively, of the vitamin E level of AIN-76 diet for rats, and 17 α-TE was the level of vitamin E in the soybean oil used per kg diet. Menhaden oil was kindly donated from Zapata Haynie Inc.(Reedville, VA, U.S.A.). Tertiary butylhydroxyquinone(Eastman Kodak, Rochester, NY,

Table 1. Composition of diets(g/kg)

Ingredients	Group			
	SH	SL	FH	FL
Starch[a]	370	370	370	370
Glucose[a]	190	190	190	190
Casein(vitmain-free)[b]	240	240	240	240
Cellulose[b]	40	40	40	40
Mineral mix*	40	40	40	40
Vitamin mix*[c] -vit. E	10	10	10	10
Choline[b]	0.5	0.5	0.5	0.5
Inositol[b]	5.5	5.5	5.5	5.5
DL-methionine[b]	2.2	2.2	2.2	2.2
Soybean oil	100	100	0	0
Menhaden oil	0	0	100	100
DL-α-tocopheryl acetate[c]**	183mg	0mg	206mg	23mg

SH : Soybean oil-high viamin E group
SL : Soybean oil-low vitamin E group
FH : Fish oil-high vitamin E group
FL : Fish oil-low vitamin E group
*As specified by American Institute of Nutrition.
**The levels of α-tocopherol of the SH and FH diets and the SL and FL diets were equivalent to 140 mg and 17 mg of α-tocopherol, respectively.
[a] from Jeil Feed Co.(Korea)
[b] from Teklad Test Diets (Madison, WI, U.S.A.)
[c] from Sigma Chemical Co.(St. Louis, MO, U.S.A.)

U.S.A.) was added to 0.02% of oil to prevent deterioration of dietary oils for storage[13]. The fatty acid composition of soybean oil and menhaden oil used in the experimental diets was analyzed by gas chromatography, and peroxidizability indices of fish oil diet and soybean oil diet calculated according to Witting and Horwitt[14] were 70 and 156, respectively. For fish oil and soybean oil used, P/S ratios were 1.35 and 3.89, and n-6/n-3 ratios, 0.09 and 7.68, respectively.

Preparation of liver microsomes

The liver homogenate in 0.154M KCl solution was centrifuged at 10,000×g for 20min and the supernatant fraction was recentrifuged. Microsomes were prepared by centrifugation at 105,000×g for 1 hour and the pellet was suspended in 40mM Tris-HCl buffer(pH 7.4) at the concentration of approximately 50mg protein/ml suspension. All the procedures were carried out at 4℃. After small aliquots were taken for the determination of protein concentration using Biuret method[15], the microsome suspensions were kept in 1.5ml Eppendorf vials and stored at -60℃.

Determination of lipid peroxide, α-tocopherol and retinol

Lipid peroxides were determined by the measurement of thiobarbituric acid-reactive substances (TBARS)[16]. α-Tocopherol and retinol were extracted from plasma or microsome suspension using hexane containing 0.025% butylated hydroxytoluene. The hexane phase was subjected to drying and the residues were dissolved in ether and methanol(1 : 3, v/v) and used for the analysis by high pressure liquid chromatography on a 10μm microBondapak column(3.9× 300mm, Waters, Milford, MA, U.S.A.) with the mobile phase methanol : water(96 : 4), and the peaks were detected by a UV detector at 292nm[17]. α-Tocopheryl acetate and retinyl acetate were used as the internal standards. Tissue vitamin E was determined by the method of Kayden et al[18]. For this purpose, 10% tissue homogenate in saline was saponified with KOH in the presence of 2% pyrogallol and extracted with hexane. Total tocopherol in hexane phase was reacted with ferric chloride and dipyridyl and its optical densitiy was measured at 520nm. For the analysis of tissue vitamin A, tissues were lyophilized, extracted with chloroform in the presence of butylated hydroxytoluene(50μg/ml), the extracts saponified with 10% ethanolic NaOH, and reextracted with hexane. HPLC conditions were identical to those used for plasma.

Statistical analysis

Results were statistically analyzed by ANOVA, the Scheffe multiple comparison test and Pearson's correlation test, using SPSS program.

Results and Disscussion

TBARS levels in tissues

Fig. 1 shows TBARS levels of plasma, liver, heart and kidney. TBARS level(MDA nmoles/mg lipid) of plasma was significantly higher in fish oil- fed rats than in soybean oil-fed rats, and vitamin E supplementation tended to decrease lipid peroxide level. However, the TBARS level expressed as per ml plasma were not different among groups. Liver TBARS of fish oil-fed rats with low vitamin E(FL) was the highest and 4 times as much as that of soybean oil-fed rats with high vitamin E(SH). FL group showed significantly higher heart and kidney TBARS levels than the other groups. Supplementation of vitamin E to fish oil reduced tissue TBARS up to the levels of soybean oil-fed groups. Microsome TBARS was the highest in the FL group(Table 2), which indicate the increase of lipid peroxidation of membranes due to chronic fish oil feeding under vitamin E deficiency[19]. A similar observation in the endothelium was reported by Alexander-North et al.[20] The endothelium exposed to polyunsaturated fatty acid became more susceptible to oxidative injury and the radical adduct formation was the largest in cells enriched with docosahexaenoic acid or eicosapentaenoic acid.

Correlations among TBARS levels of various tissues were shown in the Table 3. Plasma TBARS level was

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correlated with not only TBARS of liver($r=0.5870$, $P<0.001$), heart($r=0.5295$, $P<0.01$) and kidney($r=0.3333$, $P<0.05$) but also that of liver microsomes($r=0.3799$, $P<0.05$). The effect of dietary lipid was significant($P<0.001$) on the TBARS of plasma, whereas that of vitamin E supplementation was insignificant according to the two-way analysis of variance. TBARS levels of liver, heart and kidney were significantly influenced by both dietary lipid and vitamin E level. These results imply that plasma TBARS, which is easily measurable, may represent the status of peroxidative stress of other tissues.

Vitamin E levels in tissues

Plasma vitamin E levels(μg/ml or μg/mg lipid) were significantly increased by dietary vitamin E supplementation and the increase was over twice(Fig 2). Vitamin E levels of liver and heart seemed to increase with vitamin E supplementation, but the differences were insignificant. The vitamin E level of kidney of SH group was significantly higher than that of SL group, while there was no difference between FH group and FL group. Vitamin E level of plasma was significantly influenced not only by vitamin E supplementation($P<0.001$) but also by the dietary lipid ($P<0.05$) according to the two-way analysis of variance. However, those of other tissues were affected by only the vitamin E supplementation($P<0.01$). Microsomes of rats fed vitamin E-deficient diets(FL and SL) showed lower vitamin E level than SH and FH

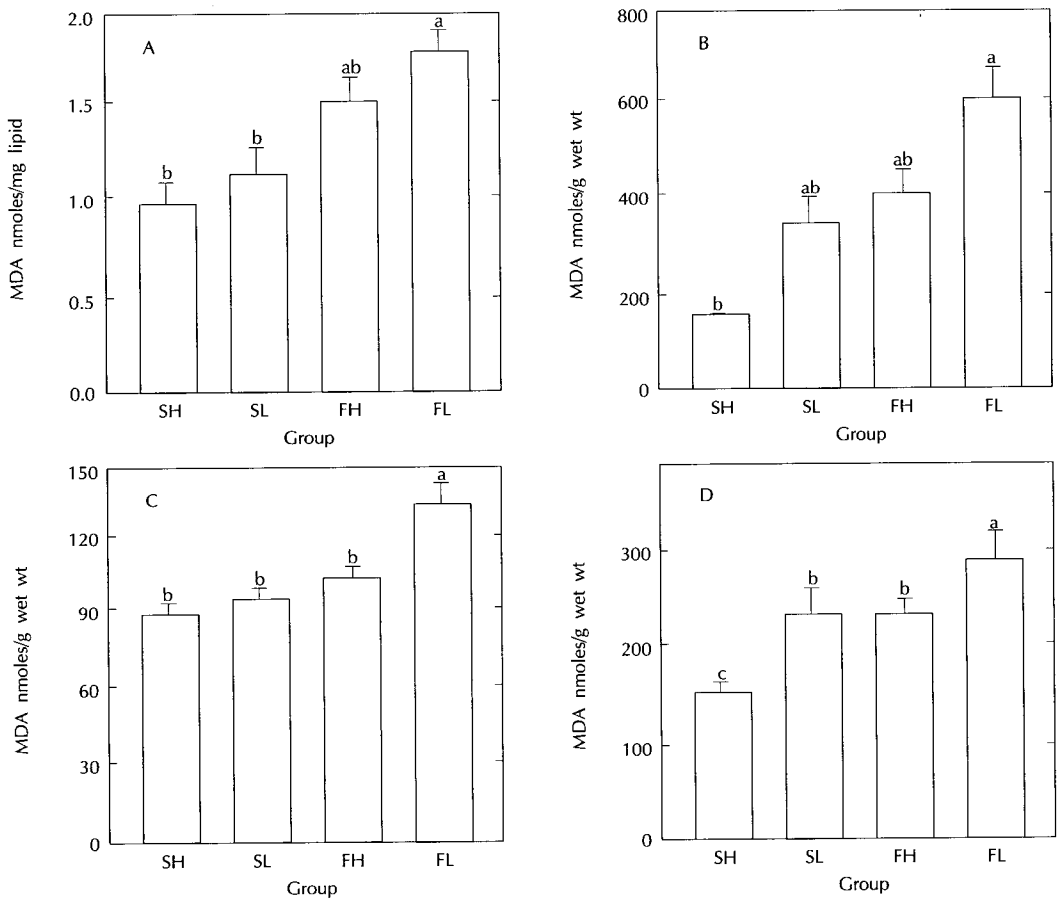

Fig. 1. TBARS levels of plasma(a), liver(b), heart(c) and kidney(d). Each bar represents mean ± SEM(n=8-10). Means not sharing a common letter are significantly different by Scheffe test($P<0.05$).

Table 2. TBARS and vitamin E levels of liver microsomes

Group	TBARS	Vitamin E
	MDA nmol/mg	μg/mg lipid
SH	6.64 ± 0.92[b]	0.520 ± 0.085[a]
SL	8.59 ± 0.84[b]	0.064 ± 0.011[c]
FH	9.33 ± 1.70[b]	0.289 ± 0.035[b]
FL	13.84 ± 2.19[a]	0.104 ± 0.015[c]

Means ± S.D.
Means not sharing the same superscript are significantly different at $P < 0.05$.

Table 3. Correlations of TBARS levels among various tissues and liver microsomes

	Liver	heart	Kidney	Liver microsomes
Plasma[1)]	0.5870***	0.5295**	0.3333*	0.3799*
Kidney	0.6411***	0.6071***		
Heart	0.5366**			
Liver microsomes	0.4208*			

1) Plasma TBARS expressed as MDA nmol/mg lipid
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

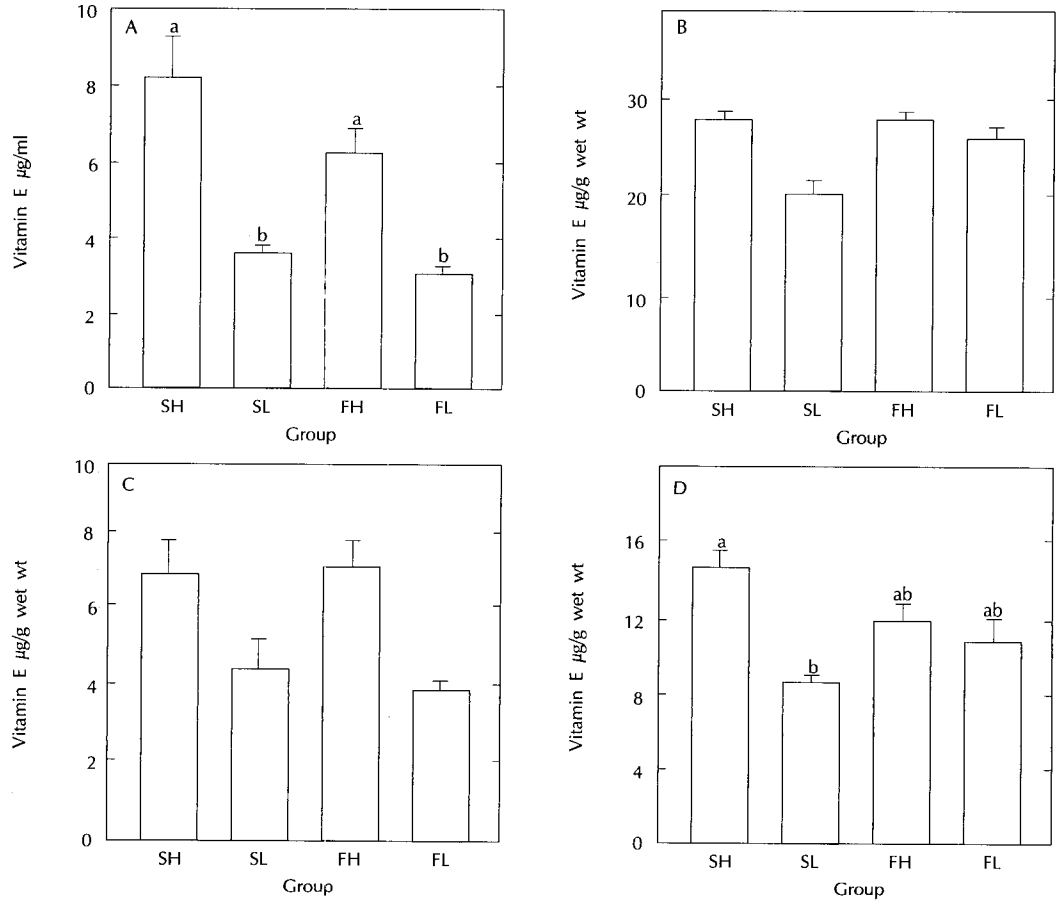

Fig. 2. Vitamin E contents of plasma(A), liver(B), heart(C) and kidney(D). Each bar represents mean ± SEM(n=8-10). Means not sharing a common letter are significantly different by Scheffe test ($P < 0.05$).

groups(Table 2).

In Table 4, plasma vitamin E level was correlated with liver($r=0.4491$, $P < 0.01$) and kidney($r=0.3126$, $P < 0.05$) vitamin E levels and liver microsome vitamin E level($r=0.3485$, $P < 0.05$), while its correlation with heart vitamin E level was not statistically significant.

Vitamin A levels in tissues

Plasma vitamin A level was significantly lower in fish oil-fed groups than in soybean-oil fed groups(Fig 3). Vitamin E supplementation did not affect the vitamin

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A level. The levels of liver, heart, kidney were not different among groups. The ingestion of n-3 fatty acids may contribute to an overall enhancement of prooxidant stress in plasma resulting in lower concentrations of endogenous antioxidants, such as vitamin E and indirectly other labile antioxidants such as retinol[8)10)]. Napoli et al[12)] reported that total vitamin A liver stores and plasma retinol were diminished during α-tocopherol deficiency. However, our results do not support the data of Napoli et al[12)].

Table 4. Correlations of vitamin E levels among various tissues and liver microsomes

	Liver	Heart	Kidney
Plasma	0.4491**	0.2376	0.3126*
Kidney	0.6419***	−0.1301	
Heart	−0.0010		
Liver microsomes	0.3485*		

*P < 0.05, **P < 0.01, ***P < 0.001

Correlations among TBARS, vitamin E and A

Plasma TBARS was negatively correlated with the vitamin E(r=-0.5763, P < 0.001) and A(r=-0.4523, P < 0.01) levels of plasma. Plama TBARS could be predicted from an equation gotten from stepwise multiple regression, TBARS(plasma ; MDA nmol/mg lipid)=-0.0826 vitamin E(plasma, μg/ml) -0.854 vitamin A (plasma, μg/ml)+2.232, in which R^2 was 0.4344.

Among the tissues examined plasma seems to be the most sensitive to the treatment such as the peroxidative susceptibility of dietary lipid as well as vitamin

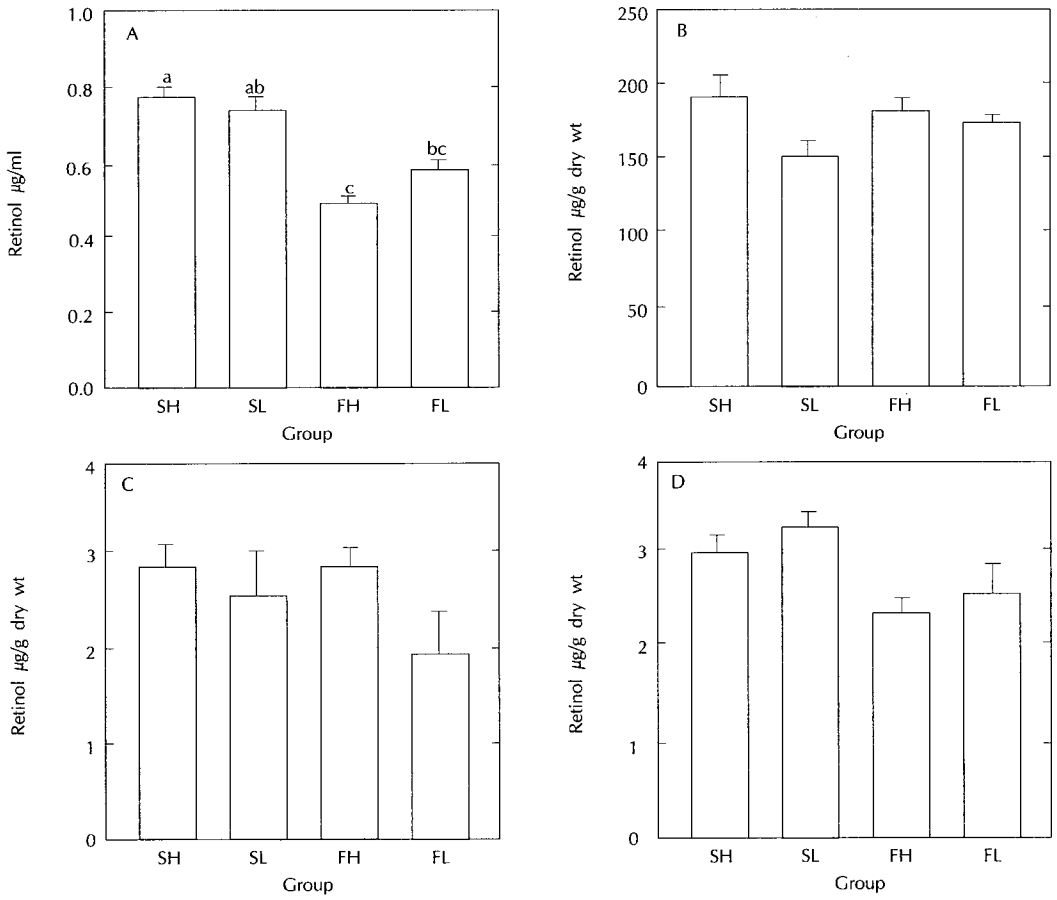

Fig. 3. Vitamin A contents of plasma(a), liver(b), heart(c) and kidney(d). Each bar represents mean ± SEM(n=8-10). Means not sharing a common letter are significantly different by Scheffe test(P < 0.05).

E supplementation. According to the work of Sheehy et al[21], in chicks fed heated sunflower oils with vitamin E deficiency, the greatest depression in α-tocopherol concentrations was evident in liver, heart, and plasma. They found that plasma and tissue α-tocopherol concentration were significantly correlated and that plasma α-tocopherol concentration and plasma TBARS level were significantly correlated. They concluded that plasma α-tocopherol was a good indicator of tissue α-tocopherol status. Our results support that plasma TBARS and vitamin E levels are reliable indicators of in vivo peroxidation.

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= 국 문 초 록 =

비타민 E 수준을 달리한 어유 또는 대두유를 먹인 흰쥐 조직의 지질과산화와 비타민 E 및 A 상태

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어유 또는 대두유에 비타민 E수준을 달리한 식이를 섭취시킨 흰쥐에서 혈장, 간, 심장, 신장 및 간 microsome의 과산화지질(TBARS)함량, vitamin E와 A 상태를 비교하므로써 과산화에 민감한 정도가 다른 두가지 지방과 비타민 E 보충에 따른 차이가 있는지, 조직의 종류에 따른 반응의 차이가 있는지를 조사하고자 하였다. 150g 내외의 Sprague-Dawley 숫쥐에게 비타민 E 결핍식이를 2주간 먹인 후, 10% 수준의 어유 또는 대두유에 17mg 또는 140mg α-tocopherol에 해당하는 비타민 E를 첨가한 실험식이를 4 주간 급여하였다. 혈장 과산화지질(MDA nmoles/mg lipid) 농도는 비타민 E 결핍시에 어유섭취군에서 대두유섭취군에 비하여 유의하게 높았으며, 비타민 E 첨가로 낮아지는 경향이었다. 간, 심장, 신장 조직도 비타민 E가 부족한 어유섭취군에서 유의하게 높았으며, 이들 조직의 과산화지질농도는 혈장 과산화지질농도와 유의한 양의 상관관계를 보였으며, 간 microsome의 과산화지질량도 혈장 과산화지질과 유의한 상관관계를 보였다. 조직의 비타민 E 수준은 비타민 E 보충으로 혈장의 비타민 E 수준은 2배이상 증가하였으나 그외의 조직은 유의한 차이가 없었는 반면에 간 microsome의 비타민 E 수준은 증가하였다. 조직의 비타민 A는 혈장에서 어유섭취군에서 유의하게 낮았으나 다른 조직에서는 차이가 없었다. 혈장의 과산화지질농도는 혈장의 비타민 E($r=-0.5763$, $P<0.001$)와 A($r=-0.4523$, $P<0.01$) 농도와 부의 상관관계를 보였다. 본연구는 비타민 E 부족시 어유섭취가 생체내 산화스트레스를 증가시켰음을 확인하였으며, 혈장 과산화지질은 생체의 산화스트레스를 반영하는 지표로 쓰일 수 있음을 보여주었다.