Effect of Dietary Vitamin E Levels on Fatty Acid Profiles and Nonenzymatic Lipid Peroxidation in the Guinea Pig Liver

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ABSTRACT: Guinea pigs were fed for five weeks with three diets containing different levels of vitamin E: LOW (but nondeficient, 15 mg of vitamin E/kg diet), MEDIUM (150 mg/kg diet), and HIGH (1,500 mg/kg diet). Dietary vitamin E supplementation did not change oxidative stress indicators in the hydrophilic compartment but increased liver α-tocopherol in a dose-dependent way and strongly decreased sensitivity to nonenzymatic *in vitro* liver lipid peroxidation. This last effect was already observed in group MEDIUM, and no further decrease in *in vitro* lipid peroxidation occurred from group MEDIUM to group HIGH. The protective effect of vitamin E against *in vitro* lipid peroxidation was observed even though an optimum dietary concentration of vitamin C for this animal model was present in the three different vitamin E diets. Both HIGH and LOW vitamin E decreased percentage fatty acid unsaturation in all phospholipid fractions from membrane origin in relation to group MEDIUM. The results, together with previous information, show that both vitamin E and vitamin C at intermediate concentrations are needed for optimal protection against lipid peroxidation and loss of fatty acid unsaturation even in normal nonstressful conditions. These protective concentrations are higher than those needed to avoid deficiency syndromes.
Lipids 31, 963–970 (1996).

Many studies suggest the involvement of oxidative damage in the etiology of important diseases such as cancer and arteriosclerosis. There is increasing epidemiological evidence (1,2), supported by some longitudinal prospective human studies (3,4), indicating that antioxidants like vitamin E and C are protective against the development of those diseases. This is probably due to their capacity to reduce oxidative damage to lipids (5,6) and other cellular macromolecules (7). Thus, there is great interest in obtaining a better knowledge about the *in vivo* effects of different dietary doses of these vitamins on tissues. Vitamin E or C is suitable for increasing antioxidant capacity safely and easily, because they are susceptible to dietary manipulation, whereas supplementation with antioxidant enzymes or GSH (which is under feedback cellular control) is not feasible in humans.

Vitamin E is considered the principal antioxidant defense against lipid peroxidation in cell membranes in mammals. Lipid peroxidation is a degradative chain reaction of oxygen radicals with unsaturated fatty acids. The most important role of vitamin E in tissues seems to be the protection of membrane polyunsaturated fatty acids (PUFA) against the deleterious effects of oxygen radicals. Specific effects of α-tocopherol that do not involve its antioxidant properties and act upon the architecture of membranes by controlling their lipid profile also have been suggested (8,9). Recent reports have also shown that vitamin E can work as a prooxidant when present in high concentrations *in vitro* (10,11); presumably *via* effects of tocopheroxyl radical.

The notion that the optimum levels of vitamins E and C to protect against *in vivo* oxidative stress are substantially higher than the recommended dietary allowance (RDA) needed to avoid deficiency syndromes is gaining experimental support. The guinea pig is the ideal laboratory animal model to perform diet-controlled *in vivo* experiments since (like higher primates) it cannot synthesize either ascorbate or α-tocopherol. We have recently shown that optimum dietary vitamin C levels in the guinea pig for protection against endogenous oxidative stress in liver are much higher than the minimum daily requirement of this animal, whereas vitamin C megadoses do not bring about further protection and are even detrimental for membrane PUFA (12). We study here the effect of three very different doses of vitamin E—from low but nondeficient levels to a very high dose—using the same guinea pig model. The study was performed at the optimum dose of vitamin C found previously (12) in order to test the efficacy of vitamin E over an adequate vitamin C background. Oxidative stress was studied both for mainly hydrophilic [glutathione redox ratio (GSH/GSSG) and protein oxidation] and lipid-dependent parameters (lipid peroxidation and fatty acid composition). Similar to what was found for vitamin C (12), intermediate dietary levels of vitamin E, sixfold higher than the minimum daily requirement,

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Abbreviations: ANOVA, analysis of variance; DNPH, 2,4-dinitrophenylhydrazine; GSH/GSSG, glutathione redox ratio; HPLC, high-performance liquid chromatography; MDA, malondialdehyde; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TBA, thiobarbituric acid; TCA, trichbroacetic acid; UI, unsaturation index; U/S, unsaturated/saturated ratio.

protect against *in vitro* liver lipid peroxidation, whereas further supplementation to high levels do not bring additional protection. Relative percentage reduction of fatty acid unsaturation in all kinds of membrane phospholipids was found both in animals receiving low or high vitamin E dietary levels compared with those receiving the medium level.

MATERIALS AND METHODS

Animals, diets, and vitamin E. Dunkin-Hartley male guinea pigs weighing 200–250 g were obtained from IFFA-CREDDO (Lyon, France). Three diets differing in vitamin E content were prepared by U.A.R. (Perpignan, France) by adding d,l-α-tocopherol acetate to the standard guinea pig diet (diet 114; U.A.R.). The vitamin E dietary content was 1,500 mg of vitamin E per kg diet (group HIGH), 150 mg vitamin E/kg (group MEDIUM), or 15 mg vitamin E/kg (group LOW). These vitamin E contents were confirmed after analysis by high-performance liquid chromatography (HPLC). Briefly, tissue samples (20–40 mg) were saponified in the presence of 0.2 mL of 15% ascorbic acid, 2 mL of ethanol containing 0.025% butylated hydroxytoluene, and 1 mL of 40% potassium hydroxide. Tocopherol was then extracted with hexane containing 0.025% butylated hydroxytoluene, the hexane was removed with N_2, the residue was redissolved in 0.5 mL ethanol, and 20 μL were injected in the HPLC. Mobil phase was 100% methanol, and the column was a C_{18} reverse phase Machery Nagel (Duren, Germany) 4.6 × 100 mm, 7 μm. The system was composed of a Waters 510 pump (Millipore Corporation, Milford, MA), a Gilson ultraviolet detector (Middleton, WI) set at 292 nm, and a Spectraphysics integrator (San Jose, CA). The basal diet contained 18.5% protein, 2.9% fat, 46.9% carbohydrates, 8.4% mineral mix, 1.4% vitamin mix, 11% humidity, and 10.9% nonnutritive bulk. The content of minerals and vitamins per kg of diet was: phosphorus, 8.6 g; calcium, 10.6 g; potassium, 12.0 g; sodium, 3.45 g; magnesium, 3.13 g; manganese, 100 mg; iron, 320 mg; copper, 26 mg; zinc, 85 mg; cobalt, 1.61 mg; vitamin A, 19,000 I.U.; vitamin D_3, 2,031 I.U.; thiamine, 22.5 mg; riboflavin, 21 mg; pantothenic acid, 123 mg; pyridoxine, 10.7 mg; menadione, 55 mg; niacin, 193 mg; folic acid, 7.3 mg; biotin, 0.275 mg; choline, 1,740 mg; myo-inositol, 250 mg; vitamin B_{12}, 0.054 mg; *p*-aminobenzoic acid, 10 mg; and vitamin C, 660 mg. This amount of dietary vitamin C was found to be optimal from the point of view of oxidative stress and fatty acid composition in a previous study (12) in the liver of male guinea pigs of the same age than those used here.

The animals were fed for five weeks the three experimental diets, and they were maintained inside aseptic air positive-pressure animal cabinets (A 130 SP; Flufrance, Cachan, France) equipped with an HEPA air filter (99.999% for particles >0.3 μm) at the inlet. At the end of the dietary regimen, the animals were sacrificed by decapitation and liver samples were immediately excised and stored at –80°C.

Nonenzymatic in vitro *lipid peroxidation.* Sensitivity to nonenzymatic *in vitro* lipid peroxidation was estimated by incubating supernatants of liver samples, obtained by centrifugation at 3,500 × *g* of liver homogenates (prepared in 50 mM phosphate buffer pH 7.4), in the presence of 0.4 mM ascorbate and 0.05 mM $FeSO_4$ for 0, 90, and 180 min at 37°C. At the end of the incubation period, the thiobarbituric acid (TBA) assay was performed by a method specially adapted to tissue extracts (13). MDA (malondialdehyde–bisdimethyl acetal; Merck, Darmstadt, Germany) was used as a standard. The results were expressed as nanomoles of MDA equivalents produced per min per g tissue and represent the sensitivity of liver lipids to an oxygen radical induced oxidative stress.

Oxidative stress in the hydrophilic compartment. In order to estimate the GSH/GSSG ratio, liver samples were homogenized in 5% trichloroacetic acid (TCA) with 0.01 N HCl, and total glutathione was measured by the spectrophotometric recycling assay (14) in the presence of 5,5′-dithiobis (2-nitrobenzoic acid), NADPH, and glutathione reductase at 412 nm. GSSG was assayed by the same method by direct derivatization of GSH homogenizing another sample from the same tissue in the presence of 12.5 mM *N*-ethylmaleimide, followed by alkaline hydrolysis of *N*-ethylmaleimide (15). GSH values were obtained after subtracting GSSG from total glutathione.

Protein carbonyl content was determined spectrophotometrically according to the 2,4-dinitrophenylhydrazine (DNPH) method (16) as previously described (12). Briefly, liver samples were treated during 15 min with 0.1% digitonin, 1 mM EDTA, phenylmethanesulfonyl fluoride (40 μg/mL), and protease inhibitors, centrifuged, the DNA was removed with 1% streptomycin (15-min incubation), and samples were centrifuged again. The supernatants were divided into two portions which were treated with 2 M HCl or 10 mM DNPH in 2 M HCl. Proteins were precipitated with 20 and 10% TCA and the pellets were washed three times with ethyl acetate/ethanol. Pellets were dissolved in 6 M guanidine hydrochloride in 20 mM potassium phosphate (pH 2.3). Each DNPH sample was scanned from 320 to 410 nm against the HCl corresponding sample, and the peak absorbance was used to calculate protein carbonyls (extinction coefficient 22,000 M^{-1} cm^{-1}). The protein concentration was read at 280 nm in the HCl sample using 6 M guanidine hydrochloride as a blank.

Fatty acid analyses. Liver fatty acids were analyzed as previously described (17). Lipids were extracted with 2-propanol that was then evaporated with N_2. The lipid fractions were separated by thin-layer chromatography on silica gel plates. The phospholipid composition was studied using chloroform/methanol/acetic acid/water (50:25:8:4, by vol) and visualized with 10% $CuSO_4$ in 8% H_3PO_4. Lipids were transesterified with 5% HCl in methanol at 75°C. The esters were extracted with *n*-pentane and saturated NaCl, evaporated under N_2 and redissolved in CS_2. Fatty acid methyl esters were analyzed on a gas chromatograph equipped with a fused silica capillary column (Supelcowax TM 10; 30 m × 0.53 mm; Supelco, Inc., Bellefonte, PA). Chromatographic conditions: carrier gas, N_2, at 60 mL/min; flame-ionization

detector with H_2 at 50 mL/min and air at 500 mL/min; injector and detector temperature, 250°C; column temperature was increased from 135 to 195°C: increase of 4° per min; from 195 to 215°C: increase of 1° per min; from 215 to 235°C: increase of 2° per min. Identification of esters was made by comparison with authentic standards (Sigma, St. Louis, MO).

Statistical analysis. Comparisons between the three vitamin E groups were analyzed by one-way analysis of variance (ANOVA). After the ANOVA, the Fisher's least significant difference test was used to analyze significance between paired groups when necessary. *In vitro* lipid peroxidation was analyzed by two-way ANOVA (for time and vitamin E group) and by one-way ANOVA (as a function of incubation time). The 0.05 level was selected as the point of minimal statistical significance in every comparison.

RESULTS

Vitamin E supplementation. Vitamin E supplementation did not affect the growth rate of the animals after either 3 or after 5 wk of treatment (data not shown). The same was true for food ingestion (data not shown). Vitamin E dietary supplementation effectively led to very different hepatic levels of vitamin E in the three groups after 5 wk of supplementation. Increasing vitamin E dietary content one order of magnitude from group LOW to group MEDIUM resulted in a 129% increase in liver α-tocopherol concentration (from 11.9 ± 1.9 to 27.2 ± 3.2 μg/g of tissue). When dietary vitamin E was further increased another order of magnitude from group MEDIUM to group HIGH, the liver α-tocopherol concentration increased an additional 196% (from 27.2 ± 3.2 to 80.4 ± 5.6 μg/g). The hepatic α-tocopherol content of the group LOW (11.9 μg/g) showed that these animals were not vitamin E-deficient.

Sensitivity to in vitro *lipid peroxidation.* No differences in lipid peroxidation were observed between the three vitamin E groups at time 0 (Fig. 1). Incubation in the presence of Fe^{++}-ascorbate significantly increased the production of lipid peroxidation products in the three groups. The rate of increase in lipid peroxidation was significantly higher in group LOW than in the other two groups showing higher liver α-tocopherol levels (Fig. 1). No significant differences between group MEDIUM and group HIGH were found at any incubation time.

We have previously shown (12) that vitamin C supplementation also decreases nonenzymatic *in vitro* lipid peroxidation in the guinea pig liver under the same experimental conditions used here (Fig. 2A). The optimum intermediate level of vitamin C supplementation found in that work, 660 mg of vitamin C/kg of diet, is the one used in the present experiment in all the vitamin E groups. Similar degrees of lipid peroxidation were observed in both works at LOW E plus MEDIUM C (Fig. 2: second bar in A and first bar in B). Although optimum vitamin C levels were used here in the three vitamin E groups, group LOW E showed higher *in vitro* lipid peroxidation values than groups MEDIUM E and HIGH E (Fig. 2B).

Oxidative stress in the hydrophilic compartment. Two indexes of oxidative stress were measured in the hydrophilic compartment, GSH/GSSG ratio, and protein carbonyls. The results obtained for both indexes were highly consistent since no significant changes were observed as a function of vitamin E dietary and hepatic concentration in any group (Table 1).

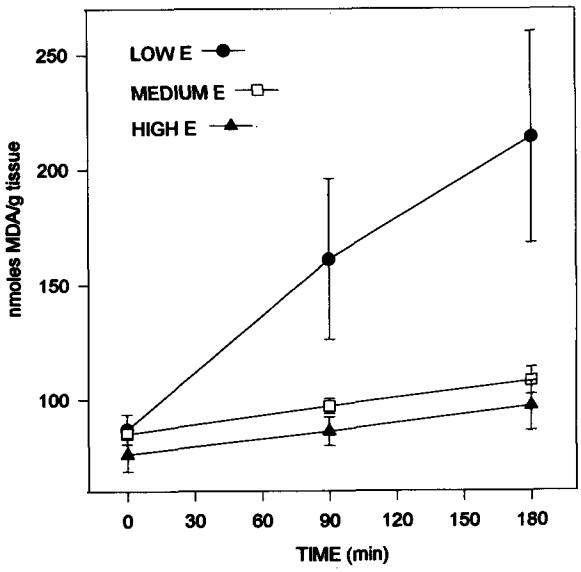

FIG. 1. Sensitivity to *in vitro* nonenzymatic (Fe^{++}-ascorbate) lipid peroxidation in the liver of guinea pigs fed with diets containing 15 (LOW E), 150 (MEDIUM E), or 1,500 (HIGH E) mg of vitamin E/kg during five weeks. Values are means ± SEM and are expressed as nmoles of malondialdehyde (MDA) equivalents/g tissue; n = seven animals per point. *In vitro* lipid peroxidation increased significantly in the three groups as a function of time [one-way analysis of variance (ANOVA); $P < 0.01$ in LOW E; $P < 0.001$ in MEDIUM E and HIGH E]. The rate of increase of liver peroxidation was significantly higher in LOW E than in the other two groups (two-way ANOVA; $P < 0.05$).

Fatty acid analyses. In the phosphatidylethanolamine fraction of liver lipids, significant changes were observed between groups MEDIUM and HIGH. Both unsaturated/saturated ratio (U/S) and the unsaturation index (UI; which takes into account the number of double bonds of each polyunsaturated fatty acid) decreased in group HIGH in relation to group MEDIUM (Table 2 and Fig. 3). This was mainly due to significant decreases in the percentage of α-linolenic (18:3n-3), arachidonic (20:4n-6), n-6 PUFA, and n-3 PUFA and to a significant increase in 16:0. The group LOW only showed a decrease in total n-6 PUFA and no significant changes were detected in any particular fatty acid. PUFA n-6 were maximum in group MEDIUM, and both HIGH and LOW vitamin E levels led to decreases in unsaturated fatty acids.

Phosphatidylcholine showed significant changes of similar character to those observed for phosphatidylethanolamine. The main changes observed occurred again in the group HIGH. n-3 PUFA, U/S ratio, UI, and linolenic acid decreased and saturated 12:0 increased in group HIGH in relation to group MEDIUM (Table 3 and Fig. 3). The unsaturated 20:2 n-6 decreased from group MEDIUM to group LOW.

In the sphingomyelin fraction, the UI, U/S ratio, n-6

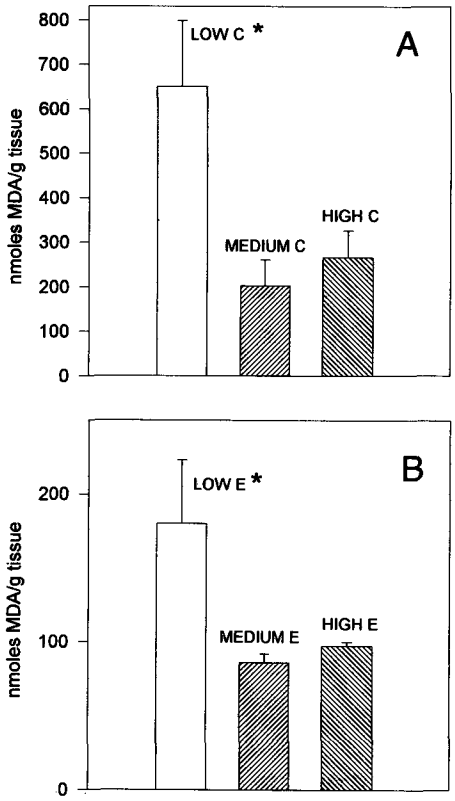

FIG. 2. Comparison of the effects on sensitivity to lipid peroxidation in the liver due to different dietary vitamin C levels in guinea pigs receiving LOW vitamin E (A) with those due to different vitamin E levels in guinea pigs receiving MEDIUM vitamin C in the diet (B). Optimum protection is obtained with the simultaneous presence of intermediate levels of both vitamins (second bar in B). Vitamin E (mg/kg) was: 15 (LOW), 150 (MEDIUM), and 1,500 (HIGH); and vitamin C (mg/kg) was: 33 (LOW), 660 (MEDIUM), and 13,200 (HIGH). Values in (B) come from Barja *et al.* (Ref. 12). Both in A and B the species, strain, body sizes, time of vitamin C or E supplementation, and lipid peroxidation conditions (90 min in the presence of ascorbate-Fe^{++}) were the same. Values are expressed as nmoles of MDA equivalents/g tissue; * = significant difference in relation to the other two groups, $P < 0.05$. See Figure 1 for abbreviation.

TABLE 1
***In Vivo* Oxidative Stress-Related Parameters in the Liver Hydrophylic Compartment of Guinea Pigs Maintained During Five Weeks in Three Diets Differing in Vitamin E Levels**

	mg of Vitamin E/kg diet		
	LOW (15)	MEDIUM (150)	HIGH (1,500)
GSH/GSSG	47.4 ± 7.4	49.1 ± 11.6	39.5 ± 2.2
Protein carbonyls (nmol/mg protein)	4.0 ± 0.9	4.7 ± 1.2	4.0 ± 1.0

[a]Values are means ± SEM from 5–6 (GSH/GSSG), or 7 (protein carbonyls) animals per group.

TABLE 2
Fatty Acid Composition (mol%) of Phosphatidylethanolamine in the Liver of Guinea Pigs Maintained During Five Weeks in Three Diets Differing in Vitamin E Levels[a]

	mg of Vitamin E/kg diet				
	LOW (15)		MEDIUM (150)		HIGH (1,500)
16:0	7.99 ± 0.52		7.64 ± 0.34	*	8.81 ± 1.01
16:1	1.02 ± 0.05		1.03 ± 0.17		1.08 ± 0.26
18:0	36.93 ± 1.59		36.08 ± 1.79		36.99 ± 1.52
18:1	5.93 ± 0.58		5.27 ± 0.58		5.48 ± 0.70
18:2n-6	30.08 ± 2.23		31.04 ± 1.06		32.16 ± 1.72
18:3n-3	2.19 ± 0.81		2.93 ± 0.67	*	1.78 ± 0.86
20:3n-6	0.37 ± 0.03		0.41 ± 0.08		0.44 ± 0.22
20:4n-6	13.05 ± 1.13		13.35 ± 1.20	*	11.39 ± 2.13
22:5n-3	1.05 ± 0.12		0.99 ± 0.14		0.95 ± 0.14
22:6n-3	0.88 ± 0.10		0.93 ± 0.18		0.89 ± 0.16
SFA	44.92 ± 1.32		43.73 ± 1.55		45.80 ± 2.31
PUFA n-6	45.70 ± 1.70	*	47.74 ± 1.61	*	45.78 ± 1.63
PUFA n-3	4.13 ± 0.91		4.85 ± 0.61	*	3.63 ± 0.92
MUFA	6.95 ± 0.60		6.31 ± 0.55		6.56 ± 0.83
U/S	1.21 ± 0.06		1.28 ± 0.08	*	1.18 ± 0.09
UI	3.06 ± 0.19		3.26 ± 0.24	*	2.92 ± 0.30
Chain length	18.06 ± 0.16		18.12 ± 0.10		18.06 ± 0.16

[a]Values are means ± SEM from seven animals per group. U/S is the ratio between Σmol% of unsaturated and Σmol% saturated fatty acids. The unsaturation index (UI) corresponds to the ratio: (Σmol% of unsaturated fatty acids × number of double bonds of each unsaturated fatty acid)/Σmol% of saturated fatty acids (SFA), PUFA n-6: Polyunsaturated fatty acids n-6; (Σmol% 18:2 + 20:3 + 20:4 + 22:4). PUFA n-3: Polyunsaturated fatty acids n-3; (Σmol% 18:3 + 22:5 + 22:6). MUFA: Monounsaturated fatty acids (Σmol% 16:1 + 18:1). Average chain length = $[(\Sigma\%Total_{14} \times 14) + (\Sigma\%Total_{16} \times 16) + (\Sigma\%Total_{18} \times 18) + (\Sigma\%Total_{20} \times 20) + (\Sigma\%Total_{22} \times 22)]/100$. Asterisks between columns describe differences between LOW E and MEDIUM E or between MEDIUM E and HIGH E. * = $P < 0.05$.

PUFA, arachidonic and linoleic acid decreased in group HIGH in relation to group MEDIUM (Table 4 and Fig. 3). Even though 16:0 was decreased in group HIGH in relation to group MEDIUM, this was more than compensated by an increase in 18:0, and saturated fatty acids (SFA) did not differ between the two groups. The only changes observed between groups MEDIUM and LOW were a decrease in n-6 PUFA and a decrease in the U/S ratio. Thus, again both low and high vitamin E levels caused a reduced percentage of unsaturated fatty acids, and the changes were more marked in HIGH than in LOW (but nondeficient) vitamin E animals.

More significant and quantitatively greater changes were observed for lysophosphatidylcholine, in which alterations followed the same pattern as in the rest of membrane phospholipid fractions. Group HIGH showed very significant decreases in n-6 PUFA, U/S ratio, UI, and arachidonic acid and important increases in 18:0 in relation to group MEDIUM (Table 5). The increase in 18:0, similarly to what was observed for sphingomyelin, compensated for the decrease in 16:0 and in this case the increase in the percentage of SFA in group HIGH reached statistical significance. Decrease in vitamin E from MEDIUM to LOW groups resulted in highly significant decreases in UI, n-6 PUFA, and arachidonic acid, and in increased 18:0, decreased 16:0, and increased total SFA. When both increases or decreases were considered, the changes observed with HIGH and LOW vitamin E levels in relation to group MEDIUM pointed always in the same direc-

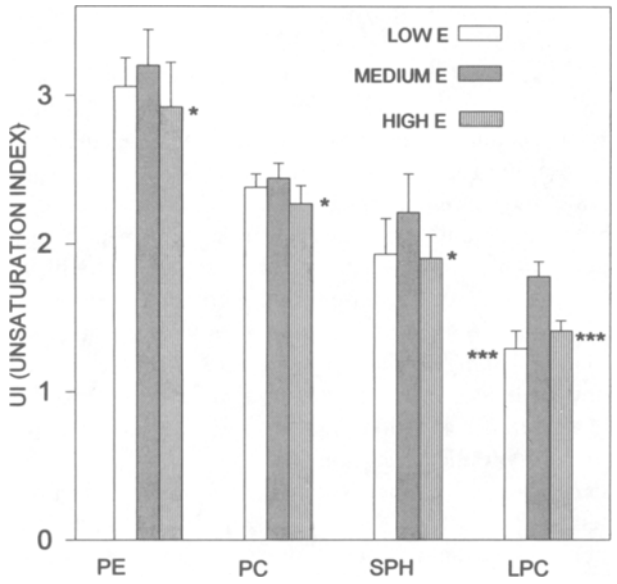

FIG. 3. Decreases in fatty acid unsaturation with both LOW and HIGH vitamin E dietary supplementation in liver guinea pig phospholipids. Vitamin E (mg/kg) was: 15 (LOW), 150 (MEDIUM), and 1,500 (HIGH). PC = phosphatidylcholine; PE = phosphatidylethanolamine; SPH = sphingomyelin; LPC = lysophosphatidylcholine; UI = unsaturation Index (see Tables 2–5). Asterisks: significant differences respect to group MEDIUM (* = $P < 0.05$; *** = $P < 0.001$).

TABLE 3
Fatty Acid Composition (mol%) of Phosphatidylcholine in the Liver of Guinea Pigs Maintained During Five Weeks in Three Diets Differing in Vitamin E Levels[a]

	mg of Vitamin E/kg diet		
	LOW (15)	MEDIUM (150)	HIGH (1,500)
12:0	0.06 ± 0.01	0.05 ± 0.01 *	0.07 ± 0.01
14:0	0.16 ± 0.02	0.14 ± 0.02	0.18 ± 0.04
16:0	15.80 ± 0.56	15.39 ± 0.87	16.44 ± 1.42
16:1	1.15 ± 0.32	0.83 ± 0.23	0.95 ± 0.26
18:0	28.44 ± 0.97	28.79 ± 0.39	29.24 ± 2.03
18:1	12.44 ± 1.11	12.91 ± 1.42	12.02 ± 0.69
18:2n-6	35.51 ± 0.59	34.98 ± 1.80	35.16 ± 1.25
18:3n-3	1.08 ± 0.44	2.00 ± 1.15 *	0.87 ± 0.13
20:2n-6	0.43 ± 0.03 *	0.49 ± 0.04	0.47 ± 0.08
20:3n-6	0.77 ± 0.13	0.80 ± 0.20	0.78 ± 0.19
20:4n-6	2.82 ± 0.40	2.97 ± 0.32	2.85 ± 0.45
22:4n-6	0.28 ± 0.03	0.30 ± 0.05	0.34 ± 0.03**
22:5n-3	0.15 ± 0.03	0.17 ± 0.02	0.16 ± 0.01
22:6n-3	0.25 ± 0.05	0.22 ± 0.03	0.21 ± 0.02
SFA	44.47 ± 1.31	44.39 ± 0.87 *	45.94 ± 1.40
PUFA n-6	40.48 ± 0.41	41.07 ± 1.54	40.03 ± 1.38
PUFA n-3	1.49 ± 0.42	2.40 ± 1.13 *	1.25 ± 0.11
MUFA	13.59 ± 1.26	13.75 ± 1.60	12.97 ± 0.81
U/S	1.23 ± 0.06	1.25 ± 0.04 *	1.17 ± 0.06
UI	2.38 ± 0.09	2.44 ± 0.10 *	2.27 ± 0.12
Chain length	17.65 ± 0.06	17.79 ± 0.07	17.71 ± 0.10

[a]Values are means ± SEM from seven animals per group. Asterisks at right of HIGH E values describe differences between HIGH E and LOW E. *$P < 0.05$; **$P < 0.01$. For other explanations and abbreviations see Table 2.

TABLE 4
Fatty Acid Composition (mol%) of Sphingomyelin in the Liver of Guinea Pigs Maintained During Five Weeks in Three Diets Differing in Vitamin E Levels[a]

	mg of Vitamin E/kg diet		
	LOW (15)	MEDIUM (150)	HIGH (1,500)
16:0	12.13 ± 2.71	10.43 ± 1.62 *	8.42 ± 0.97**
16:1	0.73 ± 0.09	0.79 ± 0.10	0.78 ± 0.17
18:0	38.97 ± 4.46	37.96 ± 3.62 *	42.66 ± 3.30
18:1	9.80 ± 1.52	10.29 ± 1.42	10.94 ± 2.18
18:2n-6	21.79 ± 2.85	24.43 ± 4.09 *	21.14 ± 1.34
18:3n-3	2.34 ± 0.50	2.76 ± 0.80	2.55 ± 0.79
20:3n-6	1.79 ± 0.50	1.82 ± 0.68	2.31 ± 0.48
20:4n-6	8.00 ± 1.39	8.74 ± 1.48 *	7.29 ± 1.04
22:0	2.25 ± 0.64	2.36 ± 0.61	2.62 ± 0.38
22:6n-3	1.03 ± 0.42	1.00 ± 0.21	1.10 ± 0.33
SFA	53.37 ± 3.73	50.76 ± 2.62	53.71 ± 3.04
PUFA n-6	33.93 ± 2.51 *	37.76 ± 3.72 *	33.31 ± 1.41
PUFA n-3	2.82 ± 0.70	2.83 ± 0.76	3.41 ± 0.48
MUFA	10.53 ± 1.47	11.08 ± 1.39	11.73 ± 2.15
U/S	0.85 ± 0.11 *	0.98 ± 0.10 *	0.86 ± 0.09
UI	1.93 ± 0.24	2.21 ± 0.26 *	1.90 ± 0.16
Chain length	17.86 ± 0.31	18.23 ± 0.14	18.13 ± 0.19

[a]Values are means ± SEM from seven animals per group. * = $P < 0.05$; ** = $P < 0.01$. For other explanations and abbreviations see Table 2.

TABLE 5
Fatty Acid Composition (mol%) of Lysophosphatidylcholine in the Liver of Guinea Pigs Maintained During Five Weeks in Three Diets Differing in Vitamin E Levels[a]

	mg of Vitamin E/kg diet		
	LOW (15)	MEDIUM (150)	HIGH (1,500)
16:0	8.80 ± 1.13 *	11.88 ± 1.87 ***	7.94 ± 0.76
18:0	53.46 ± 3.04 ***	44.96 ± 2.95 ***	52.52 ± 1.34
18:1	9.23 ± 0.83	8.92 ± 1.16	9.69 ± 0.91
18:2n-6	21.48 ± 1.95	22.42 ± 1.63	22.55 ± 1.44
20:4n-6	7.07 ± 0.85 ***	11.91 ± 1.09 ***	7.65 ± 0.35
SFA	62.27 ± 1.94 ***	56.85 ± 2.00 *	60.47 ± 1.83**
PUFA n-6	28.56 ± 2.47 ***	34.33 ± 1.85 ***	30.20 ± 1.46
MUFA	9.23 ± 0.83	8.92 ± 1.16	9.69 ± 0.91
U/S	0.60 ± 0.04	0.76 ± 0.04 ***	0.66 ± 0.04*
UI	1.29 ± 0.12 ***	1.78 ± 0.10 ***	1.41 ± 0.07*
Chain length	17.97 ± 0.03	18.01 ± 0.23	18.06 ± 0.23

[a]Values are means ± SEM from seven animals per group. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$. For other explanations and abbreviations see Table 2.

tion: decreases in the degree of total fatty acid unsaturation (Fig. 3).

The changes observed in the triglyceride fraction were totally different from those observed for phospholipids mainly derived from membrane fractions. Group HIGH showed increases in 14:0 and 16:0 and a decrease in 18:0 in relation to group MEDIUM, and an increase in the percentage of SFA in relation to group LOW (Table 6). Group LOW showed higher 18:2 n-6 and n-6 PUFA and lower 18:0 than group MEDIUM. Neither the U/S ratio nor the UI showed changes as a function of vitamin E levels (Table 6).

TABLE 6
Fatty Acid Composition (mol%) of Triglycerides in the Liver of Guinea Pigs Maintained During Five Weeks in Three Diets Differing in Vitamin E Levels[a]

	mg of Vitamin E/kg diet		
	LOW (15)	MEDIUM (150)	HIGH (1,500)
12:0	0.83 ± 0.20	0.94 ± 0.04	0.88 ± 0.13
14:0	0.34 ± 0.06	0.35 ± 0.04 **	0.43 ± 0.04
16:0	27.68 ± 2.80	26.31 ± 1.75 *	30.98 ± 4.45*
16:1	0.80 ± 0.17	0.95 ± 0.06	0.88 ± 0.10
18:0	8.95 ± 0.44 ***	11.79 ± 1.23 **	9.27 ± 1.53
18:1	22.14 ± 0.46	22.14 ± 1.44	21.47 ± 1.76
18:2n-6	30.78 ± 0.90 ***	28.91 ± 0.31	29.20 ± 3.40
18:3n-3	4.72 ± 0.77	4.85 ± 0.80	5.20 ± 0.25
20:4n-6	1.46 ± 0.30	1.54 ± 0.11	1.53 ± 0.12
22:6n-3	0.73 ± 0.17	0.68 ± 0.04	0.61 ± 0.09
SAF	37.81 ± 2.42	39.40 ± 2.93	41.57 ± 3.09*
PUFA n-6	36.96 ± 1.07 *	35.30 ± 1.12	35.94 ± 3.65
PUFA n-3	5.46 ± 0.86	5.53 ± 0.77	5.81 ± 0.31
MUFA	22.94 ± 0.64	23.10 ± 1.40	22.35 ± 1.87
U/S	1.61 ± 0.12	1.50 ± 0.15	1.43 ± 0.22
UI	2.89 ± 0.23	2.70 ± 0.27	2.58 ± 0.41
Chain length	15.53 ± 0.38	15.04 ± 0.24	15.76 ± 0.16

[a]Values are means ± SEM from seven animals per group. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$. For other explanations and abbreviations see Table 2.

The mean chain length was not modified in any lipid fraction as a result of vitamin E treatment.

DISCUSSION

In the present study, we have used three different levels of vitamin E in the diet to obtain guinea pigs with three different hepatic concentrations of vitamin E. Animals in the LOW vitamin E group ingested 0.6–0.75 mg of vitamin E per day, a dose very close to the minimum daily vitamin E requirement for long-term maintenance of the growing guinea pig, 1 mg/d (18,19). Animals in this group LOW have low tissue vitamin E levels, but they cannot be considered vitamin E-deficient. Animals in group MEDIUM are in the normal range used for routine maintenance of guinea pig, receiving an amount of vitamin E more than sixfold higher than the minimum daily requirement. Finally, group HIGH was designed to clarify the effects of supplementing the diet with levels of vitamin E 65-fold higher than the minimum daily requirement on hydrophilic and lipid oxidative stress markers and fatty acid profiles in the guinea pig liver.

In spite of the presence of widely different hepatic α-tocopherol contents, neither GSH/GSSG ratio or protein carbonyls varied. The reduced and oxidized forms of glutathione are present in the hydrophilic compartment, and the same is true for a great part of tissue proteins. That result is consistent with the function of vitamin E as an important antioxidant in membranes but not in the hydrophilic cellular compartment.

Contrasting with this lack of effect in the hydrophilic compartment, the increase in liver α-tocopherol from group LOW to group MEDIUM strongly inhibited nonenzymatic (induced by Fe^{++}-ascorbate) *in vitro* lipid peroxidation. This is consistent with an important antioxidant role of vitamin E in the membrane cellular compartment. Further increases in liver α-tocopherol to group HIGH did not afford additional protection in our system, perhaps because under these conditions ascorbate eventually becomes limiting. Some reports have shown similar results for laboratory rodents. A diet with 180 mg of vitamin E/kg decreased lipid peroxidation in relation to a diet with 86 mg/kg in rat liver after almost one month, whereas 1,400 mg/kg did not bring further protection (20). Vitamin E dietary supplementation also decreased susceptibility to lipid peroxidation in rat liver (21,22), and peroxidation of rat liver microsomes induced by Fe^{++}-ascorbate (23). No decreases in liver peroxidation were described in mice treated for 7 wk with 200 mg of vitamin E/kg vs. a deficient diet, but tissue vitamin E levels were not measured (24). The reason for the lack of differences in *in vitro* lipid peroxidation between groups MEDIUM and HIGH is not known, but the possibility exists that most ascorbate is consumed during the first part of prolonged incubation. It is possible that the amount of oxidative stress generated by our incubation system was not high enough to produce a vitamin E depletion which could discriminate between MEDIUM and HIGH vitamin E levels.

Previous work from our laboratory showed that vitamin C supplementation decreases nonenzymatic *in vitro* lipid peroxidation in LOW E guinea pig liver under the same experimental conditions used here. In the present work, we show that vitamin E protects from *in vitro* lipid peroxidation in spite of the use—in the three vitamin E groups—of the optimum (intermediate) vitamin C concentration found in our earlier work (12). Thus, optimum protection from hepatic *in vitro* lipid peroxidation in the guinea pig needs the simultaneous supplementation with both vitamins (C and E) at intermediate levels. This is consistent with the proposal of a cooperation between both vitamins *in vivo*, vitamin C reducing α-tocopheroxyl radical back to vitamin E (25,26).

Unsaturated fatty acids are highly sensitive molecules to free radical damage in cells. This sensitivity increases as a function of the number of double bonds of each individual fatty acid (27). Few works have addressed the problem of the possible effects of dietary vitamin E on tissue fatty acid profiles (28), as we have here in liver, and to our knowledge none has been performed in guinea pigs. A decrease in fatty acid unsaturation in liver microsomes of rat pups fed a vitamin E-deficient diet during 9 mon, the mothers receiving the same diet during gestation, has been recently described (29). In this work, liver vitamin E decreased to levels much lower than in our work (0.8 μg/g liver) and decreases in unsaturated 16:1, 18:1, 18:2, 20:3, and increases in 18:0 and total SFA were found in the vitamin E-deficient group. No group supplemented with vitamin E megadoses was included in that study. Other authors also have found decreases in unsaturation in liver microsomes and mitochondria in vitamin E-deficient rats

(30). At the other end of the spectrum, 2,000 mg of dietary vitamin E/kg diet during 6 wk decreased UI, 20:4, and 22:6 and increased 18:2 in the liver of spontaneously hypertensive rats (31). No group with low E levels was included in this work, only phosphatidylcholine was studied, and SFA were not measured. Other authors did not find changes in fatty acid unsaturation in rat tissues after feeding low vitamin E diets (32). Problems in study design may explain differences in results reported. As an example, no changes in liver PUFA were found after feeding Swiss mice diets with 0, 50, or 150 mg of vitamin E/kg, but no changes in liver vitamin E were found among the three dietary groups (33). Clearly, the lack of effect on PUFA in this study was associated with a lack of change in liver vitamin E.

In our present work, we have shown that both LOW and HIGH dietary and hepatic vitamin E levels were associated with a significantly lower fatty acids percentage of unsaturated, and maximum unsaturation occurs at intermediate vitamin E levels (Fig. 3). The LOW E effect is observed even in animals in which tissue vitamin E concentrations have not fallen to deficiency levels. Quantitatively greater effects were observed at HIGH than at LOW dietary vitamin E. When significant changes were observed, they occurred in the same direction in all the lipid fractions except for the triglycerides, indicating that the protective antioxidant role of vitamin E at intermediate levels operates mainly in membrane lipids. Triglycerides are deposited in hepatic cells surrounded by other molecules like phospholipids or cholesterol. This may afford them protection against oxidative stress, thus explaining their minor changes after vitamin E supplementation in comparison with those shown by phospholipids. The changes observed in phospholipids always occurred in the same direction from the group MEDIUM to the other two groups: decreases in the percentage of unsaturated fatty acids.

The decrease in unsaturation at low vitamin E levels could be predicted from prior work on the antioxidant role of this vitamin in the lipid membrane environment. Concerning the relative reduction of unsaturation at high vitamin E doses, various possibilities arise. It has been shown that α-tocopherol can have a prooxidant effect in chemical (10) and low density lipoprotein systems (11) *in vitro*. The prooxidant effect has been attributed to the reaction of tocopheroxyl radical (the oxidized free-radical form of vitamin E) with unsaturated lipids and lipid hydroperoxides, forming alkyl and peroxyl lipid radicals (10). This prooxidant effect does not occur if sufficiently high concentrations of antioxidants like vitamin C or ubiquinol-10, which reduce tocopheroxyl radical to vitamin E, are present in the *in vitro* system (11). This explanation would be consistent with our *in vitro* results, since the dietary vitamin E level of the group HIGH (1,500 mg/kg) surpassed that of vitamin C (660 mg/kg) in the present work. Nevertheless, no increases in oxidative stress markers (GSH/GSSG and protein carbonyls) were found in group HIGH. Furthermore, only percent, not absolute, fatty acid values were measured. Thus, the decreases in percent unsaturated fatty acids can be due to increases in SFA instead of to decreases in absolute amounts of unsaturates. Other alternatives are also possible. Effects of vitamin E on the synthesis of unsaturated fatty acids cannot be discarded since it has been shown that Δ-9 desaturase activity is decreased both by vitamin E-deficient and by vitamin E- supplemented diets in rat liver microsomes (34) and that high vitamin E concentrations (20-fold the control level) decrease Δ-6 desaturase in rat liver (35). Finally, the decrease in unsaturation in group HIGH also can be due to an inhibitory effect of high doses of α-tocopherol on phospholipase activity (36). This would decrease the elimination from the membranes of lipid hydroperoxides, known propagators of lipid peroxidation. In any case, the results show decreases in the percentage of unsaturated membrane fatty acids at both too low and too high dietary vitamin E doses in the liver *in vivo*.

A decrease in liver fatty acid unsaturation with both too low (33 mg/kg) and too high (13,200 mg/kg) dietary vitamin C levels has been found in our laboratory in the same guinea pig model used here, and higher unsaturation was observed again at intermediate (660 mg/kg) vitamin C dietary levels (12). These intermediate ascorbate levels were also enough to protect against lipid peroxidation. For both antioxidant vitamins (E and C), an intermediate dietary vitamin level exists at which protection from lipid peroxidation and a percentage of unsaturated higher fatty acids is observed in the liver. That level is 40- (vitamin C) or 6-fold (vitamin E) higher than the minimum daily requirement needed to avoid deficiency syndromes. This is consistent with the idea that optimum dietary levels of vitamin E and C are probably higher than the current RDA, whereas megadose levels can alter membrane fatty acid profiles in the same direction as marginal antioxidant vitamin deficiency.

ACKNOWLEDGMENTS

This work was financially supported by grant n°93/0145E from the National Research Foundation of the Spanish Ministry of Health (FISss). Predoctoral fellowships were received by C. Rojas and S. Cadenas (F.P.I., Ministry of Education) and a postdoctoral fellowship by R. Pérez-Campo (FISss).

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[Received July 26, 1995, and in final revised form June 10, 1996; Revision accepted July 5, 1996]