Effect of oral supplementation with D-α-tocopherol on the vitamin E content of human low density lipoproteins and resistance to oxidation[1]

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Abstract Twelve clinically healthy subjects participated in a vitamin E supplementation study. Eight were given daily dosages of 150, 225, 800, or 1200 IU RRR-α-tocopherol for 21 days (two persons per dose) and four received placebo. Prior, during, and after the supplementation period, α-tocopherol, γ-tocopherol, and carotenoids were determined in plasma and low density lipoprotein (LDL). The maximum levels of α-tocopherol were 1.7- to 2.5-times the baseline values in plasma and 1.7- to 3.1-times in LDL. A high correlation existed between α-tocopherol in plasma and LDL. γ-Tocopherol significantly decreased in plasma and LDL during vitamin E supplementation. No significant influence on the lipoprotein and lipid status and carotenoid levels of the participants occurred throughout the supplementation. The resistance of LDL against copper-mediated oxidation was also measured. The oxidation resistance of LDL was significantly higher during vitamin E supplementation. However, the efficacy of vitamin E in protecting LDL varied from person to person. The statistical evaluation of all data gave a correlation of $r^2 = 0.51$ between α-tocopherol in LDL and the oxidation resistance as measured by the length of the lag-phase preceding the oxidation of LDL. No association was seen between levels of carotenoids and vitamin E in plasma and LDL. The present study clearly shows that in humans the oxidation resistance of LDL can be increased by vitamin E supplementation. —**Dieber-Rotheneder, M., H. Puhl, G. Waeg, G. Striegl, and H. Esterbauer.** Effect of oral supplementation with D-α-tocopherol on the vitamin E content of human low density lipoproteins and resistance to oxidation. *J. Lipid Res.* 1991. **32:** 1325–1332.

Supplementary key words carotenoids • lipid peroxidation • oxidized LDL

Several lines of evidence indicate that the initiation of atherosclerosis is related to free radical processes, lipid peroxidation, and oxidative modification of low density lipoprotein (LDL) (1). Oxidized LDL is recognized by the scavenger receptor of macrophages and is able to convert them to lipid-loaded foam cells that are found in atherosclerotic plaques (2). In vitro LDL can be oxidized by a variety of cultured cells (endothelial cells, smooth muscle cells, monocyte-macrophages) and transition metal ions such as Cu^{2+}. The properties of copper-oxidized LDL are very similar if not identical to cell-oxidized LDL. Recent studies have shown that oxidative modification of LDL also occurs in vivo (3). Polyclonal and monoclonal antibodies that recognize epitopes generated during oxidation of LDL have been raised and it has been shown by immunostaining that such forms of LDL are in fact present in atherosclerotic lesions (4, 5).

Oxidation of LDL is a free radical process in which the polyunsaturated fatty acids of LDL are degraded in a lipid peroxidation process to a variety of highly reactive products such as lipid hydroperoxides, aldehydes, and others (for review see reference 6). Some of these products are cytotoxic and can lead to injury of the endothelial layer of blood vessels. LDL is protected against oxidation by its endogenous antioxidants, e.g., vitamin E, carotenoids, and others (7). Oxidation of polyunsaturated fatty acids in LDL is preceded by a sequential loss of these antioxidants in the sequence: α-tocopherol, γ-tocopherol, lycopene, and β-carotene (8). Our laboratory as well as others have been able to demonstrate (9, 10) that rapid oxidation of LDL can only commence when it is depleted of these antioxidants. However, there exists a strong donor-dependent variation in the oxidation resistance of LDL (11).

Several possibilities are available to follow the oxidation of LDL. One can measure the increase of aldehydes (e.g., malondialdehyde), lipid hydroperoxides, conjugated dienes, fluorescence of apolipoprotein B or LDL lipids, relative electrophoretic mobility, or the decrease of antioxidants and polyunsaturated fatty acids (6). A very convenient method to measure the rate of oxidation in LDL is to follow the oxidation continuously by monitor-

Abbreviations: LDL, low density lipoprotein; HPLC, high performance liquid chromatography; PUFA, polyunsaturated fatty acid.

[1]A part of this study has been presented at the 356th Meeting of the Biochemical Society in Aberdeen, UK, 1990 (12).

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ing the change of the UV absorbance at 234 nm (8). This absorbance develops in LDL during oxidation through the formation of lipid hydroperoxides with conjugated double bonds.

Previous work in our laboratory has focused on the determination of the efficiency of vitamin E in protecting LDL against oxidation. α-Tocopherol is the main antioxidant in LDL. One LDL particle contains on the average six molecules of α-tocopherol. We have made several attempts to enrich LDL in α-tocopherol in vitro and to elucidate in this way its effect on the resistance against oxidation (9, 12, 13).

We now report the results of an ex vivo study in which the oxidation resistance of LDL from volunteers supplemented with various doses of vitamin E was determined in vitro. The naturally occuring free RRR-α-tocopherol was used since this has been described as the most active tocopherol species (14).

MATERIALS AND METHODS

RRR-α-Tocopherol

Soft capsules filled with 75 or 400 IU of RRR-α-tocopherol, respectively, in wheat germ oil were provided by Henkel KGa.A. (Düsseldorf, FRG). One mg RRR-α-tocopherol is equivalent to 1.49 IU. Identical placebo capsules containing wheat germ oil only were also provided. All capsules were coated with clear soft gelatin and kept in dark glass bottles until use.

Study and subjects

Twelve healthy volunteers (male and female between 20 and 30 years of age) participated in the study. They were asked to take two or three capsules of RRR-α-tocopherol daily, together with their main meal, for 21 days. The study was performed as a single blind trial; the participants did not know which dose they received. The group was divided into four subjects who received placebo and two subjects each receiving 150, 225, 800, or 1200 IU RRR-α-tocopherol as a single dose per day. The subjects had no limitations in living and food habits during the experiment except they were asked to avoid the intake of vitamins (except those occurring in their normal diets).

Blood samples (20 ml) were taken after overnight fasting into EDTA (0.1% final concentration) 2 days prior to the supplementation (day −2), on days 3, 5, 10, 12, and 18 during the supplementation, and 6 days after (day 26) the end of the supplementation period. On day −2 and day 26 the whole lipoprotein profile (total lipids, chylomicrons, LDL, VLDL, HDL; total cholesterol, triglycerides, phospholipids, and lipid phosphorus), liver enzymes (GOT, GPT, AP, YGT, CHE), and red and white blood cell counts of the volunteers were determined.

α- and γ-tocopherol were measured in the freshly prepared plasma and low density lipoproteins (LDL) of all subjects at the seven time points mentioned above by HPLC-methods as previously described (15, 16). The amount of carotenoids (β-carotene, lycopene, cryptoxanthine, and luteine/zeaxanthine) was also determined in plasma and LDL at the same time points using a slightly modified method of Bieri, Brown, and Smith (17). Acetonitrile–dichloromethane–methanol 67:19:14 was used as mobile phase with a flow rate of 1.3 ml/min on a Spherisorb 5 μm ODS 2 column. The extracts used for antioxidant measurements were kept at −20°C under nitrogen and measured within 6 h.

Preparation of LDL

LDL was immediately prepared from EDTA plasma of all samples by density gradient ultracentrifugation for 22 h (Beckman, SW 41 rotor). Three ml of plasma was adjusted to a density of 1.22 g/ml with potassium bromide and a gradient with densities of 1.080, 1.050, and 1.005 g/ml, respectively, was used. All density solutions were prepared with potassium bromide and contained 1 mg/ml of EDTA. Before the oxidation experiments, EDTA and salts were removed from LDL by gel filtration on Sephadex PD 10 columns (Sephadex G25M, Pharmacia, bed volume 9 ml). Because even traces of EDTA would interfere with the determination of the lag-phase in our oxidation experiments (see below), the separation was repeated, i.e., each LDL fraction obtained from the first Sephadex separation was separated a second time in the same way.

Total cholesterol was determined in plasma and LDL fractions with the CHOD PAP reagent of Boehringer Mannheim.

Determination of the oxidation resistance (lag-phase) of LDL

The resistance of all LDL samples against copper-mediated oxidation was measured photometrically (Ultraspec II, LKB) by following the change of the absorption of the conjugated dienes as previously described (8). Briefly, LDL (80 μg total cholesterol, which corresponds to about 0.25 mg total LDL/ml) in oxygenated phosphate-buffered saline (10 mM, pH 7.4) was supplemented with 3.0 μM Cu^{+2} (chloride) as a prooxidant. The oxidation was followed on line at 234 nm at room temperature. The intercept with the x-axis was defined as "lag-phase" of the particular LDL.

Unless otherwise indicated, all chemicals were from Sigma.

RESULTS

Liver enzymes, blood cell counts, and lipoprotein status

Liver enzymes and red and white blood cell counts of all participants of the study were in the normal range and no significant changes were measured 6 days after termination of vitamin E suppementation.

All participants except one (code: 7, 225 IU; code numbers refer to the table) had a normal status of lipoproteins at day −2 and no significant change was observed in the measurements on day 26. Changes in total lipids on day 26 as comapred to day −2 were less than ± 10% in eight participants. Two participants (code: 3, placebo; 9, 800 IU) had a decrease in total lipids of 19%, due mainly to a decrease in total cholesterol, and two participants had an increase of total lipids of 20% and 48% on day 26 compared to day −2 (code 6, 150 IU; and the hyperlipidemic participant code 7, 225 IU).

Total cholesterol in plasma was measured at all seven time points throughout the study. There were no significant alterations in cholesterol levels either in the placebo or in the experimental group (**Table 1**).

The triglyceride values of ten participants were in the normal range before the study (day −2), but one participant (code: 7, 225 IU) had a high level of 320 mg/dl and another a low level of 38 mg/dl (code: 11, 1200 IU). There have been reports in the literature that supplementation with megadosages of vitamin E may cause an elevation of serum triglyceride levels (18). The triglyceride values that we measured after termination of the study were all, except one, slightly increased (<20%) compared to the initial values on day −2. One participant (code: 10, 800 IU) had an initial triglyceride value of 159 mg/dl which increased to 299 mg/dl. But this might rather have been caused by his diet on the days before the last blood sampling since there were no limitations concerning diet as mentioned in the Methods section.

α-Tocopherol in plasma

Plasma tocopherol levels before supplementation were in the normal standard range for healthy adults (19). There was no significant change of α-tocopherol in the placebo group during the study. The mean value of all control determinations (all values on day −2 plus all placebo values, n = 36) was 23.44 ± 3.86μM. During the

TABLE 1. Influence of RRR-α-tocopherol supplementation on cholesterol and antioxidants in human plasma and antioxidants and oxidation resistance of LDL

		Antioxidants in plasma						
Dose	Code	β-Carotene	Lycopene	Cryptoxanthine	Zea. - Lut.	Cholesterol	α-Tocopherol	γ-Tocopherol
IU		*nM*	*nM*	*nM*	*nM*	*mg/100 ml*	*μM*	*μM*
0	1-4	783/ 190-1290	223/ 60 -490	340/ 140- 630	218/ 90-390	170/ 132-215	23.3/ 16.3-27.8	2.13/ 1.18-5.01
150	5	350/ 210- 320	380/ 140- 250	350/ 200- 420	620/ 270-380	225/ 204-230	30.5/ 35.0-49.4	1.98/ 0.00-1.18
150	6	300/ 180- 240	330/ 160- 280	190/ 100- 160	220/ 150-170	172/ 156-180	19.0/ 24.1-32.7	1.37/ 0.00-0.78
225	7	350/ 230- 390	270/ 190- 350	380/ 360- 570	220/ 130-220	222/ 211-251	36.7/ 54.7-69.7	3.10/ 0.41-1.77
225	8	880/ 500-1060	230/ 220- 420	690/ 510- 690	260/ 270-390	183/ 164-189	22.9/ 34.4-43.8	2.43/ 0.00-0.70
800	9	760/ 370- 660	90/ 90- 120	160/ 90- 140	400/ 150-250	227/ 194-236	22.9/ 36.8-49.8	2.42/ 0.00-0.98
800	10	370/ 180- 280	170/ 120- 210	330/ 160- 620	330/ 130-200	250/ 207-237	30.9/ 47.5-62.9	2.33/ 0.00-0.78
1200	11	690/ 430- 720	200/ 150- 210	1110/ 780-1230	310/ 200-350	177/ 179-200	27.2/ 43.9-69.3	2.64/ 0.00-0.73
1200	12	1610/1150-1820	310/ 180- 260	470/ 470- 630	130/ 100-170	202/ 195-233	26.8/ 57.6-90.3	2.31/ 0.00-0.74
		Antioxidants in LDL						
Dose	Code	β-Carotene	Lycopene	Cryptoxanthine	Zea. - Lut.	Lag-Phase	α-Tocopherol	γ-Tocopherol
IU		*pmol/mg*	*pmol/mg*	*pmol/mg*	*pmol/mg*	*min*	*nmol/mg*	*nmol/mg*
0	1-4	178/ 43-262	54/ 11-101	49/ 18- 79	19/ 0-30	74/ 56- 86	2.82/ 2.11-4.23	0.24/0.09-0.50
150	5	60/ 28- 57	69/ 18- 52	45/ 20- 64	40/ 20-34	53/ 60- 80	2.72/ 3.57-4.56	0.16/0.00-0.13
150	6	65/ 28- 59	76/ 36- 70	28/ 16- 29	18/ 11-19	65/ 59- 80	2.71/ 3.40-3.77	0.14/0.00-0.09
225	7	54/ 47- 62	46/ 41- 65	49/ 45- 63	19/ 11-20	57/ 70- 95	2.81/ 3.37-6.57	0.26/0.03-0.12
225	8	191/ 106-173	49/ 43- 77	94/ 68- 80	22/ 22-31	75/ 115-150	2.72/ 4.01-4.86	0.27/0.04-0.07
800	9	123/ 54- 89	24/ 12- 18	17/ 10- 17	28/ 11-24	44/ 63- 75	2.97/ 3.84-4.13	0.28/0.00-0.08
800	10	61/ 23- 41	28/ 17- 22	41/ 18- 70	21/ 9-14	60/ 58- 77	2.87/ 3.87-4.78	0.21/0.00-0.05
1200	11	179/ 84-119	60/ 29- 50	192/ 97- 134	32/ 16-23	75/ 118-170	3.36/ 4.81-10.43	0.30/0.05-0.07
1200	12	360/ 211-299	74/ 33- 51	65/ 44- 66	11/ 7- 9	69/ 110-128	2.78/ 5.16-7.55	0.23/0.03-0.04

Twelve subjects received RRR-α-tocopherol, as indicated, for 21 days. The following parameters were measured 2 days before (day −2), five times during, and 6 days after vitamin E supplementation: α-tocopherol and γ-tocopherol and carotenoids in plasma and LDL by HPLC, total cholesterol enzymatically in plasma, and the resistance of LDL against oxidation (lag-phase) by recording the UV absorbance at 234 nm. Values before the slash express the initial values at day −2; values after the slash express the minimum and maximum during supplementation time (for each individual of the experimental group and the mean of four subjects in the placebo group).

21 days of α-tocopherol supplementation, the participants had significantly elevated levels of α-tocopherol in plasma as compared to day −2 (Table 1). The peak values were 246 and 250% of the initial values for the highest supplemented dose of 1200 IU, 204 and 218% for the dose of 800 IU, 190 and 192% for the dose of 225 IU, and 162 and 173% for the lowest administered dose of 150 IU. The elevated levels of α-tocopherol in plasma rapidly decreased during the week after the termination of treatment. In five participants they returned to the initial levels. They had been supplenented with 150 and 1200 IU and one with 225 IU α-tocopherol (code:8). The participant with the high triglyceride value (code: 7, 225 IU) had a 30% higher α-tocopherol level in his plasma on day 26 than on day −2. The values of the two participants that had received 800 IU during the study were still 40% higher on day 26.

Fig. 1 shows the mean of vitamin E levels (α- and γ-tocopherol) during the 21 days of supplementation versus the dietary intake of RRR-α-tocopherol. In the supplemented subjects γ-tocopherol was almost negligible (see below).

γ-Tocopherol in plasma and LDL

The mean values of γ-tocopherol for the placebo group (n = 28) were 2.48 ± 0.57 μM in plasma and 0.24 ± 0.06 nmol/mg LDL. The participant with the highest γ-tocopherol values (code: 3) was a vegetarian. He received placebo capsules. The mean of his γ-tocopherol was 3.26 ± 1.0 μM in plasma and 0.36 ± 0.10 nmol/mg LDL. γ-Tocopherol rapidly decreased in plasma and LDL of all participants during the time of α-tocopherol supplementation (Table 1) as reported also by other groups

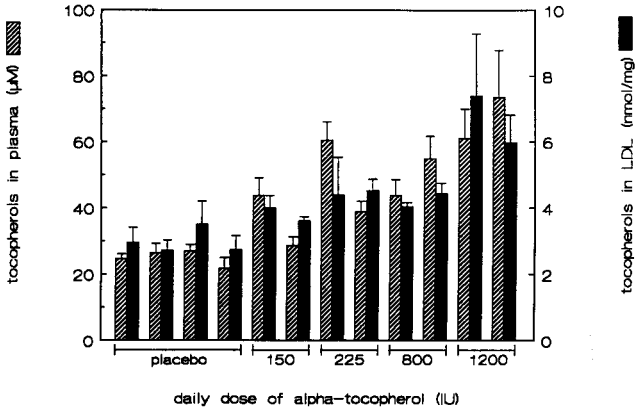

Fig. 1. Effect of dietary intake of RRR-α-tocopherol on the concentration of vitamin E in human plasma and LDL. Tocopherols (α- and γ-) were determined by HPLC in freshly prepared plasma and LDL before (day −2), five times during (on days 3, 5, 10, 12, and 18), and 6 days after oral supplementation with RRR-α-tocopherol. Values express the mean ± SD (sum of α- and γ-tocopherol) during supplementation time from each participant of the study.

for this isomer in plasma (20). γ-Tocopherol levels rapidly increased again in plasma and LDL after termination of the supplementation. Only in one individual who had received 800 IU (code: 10) did the γ-tocopherol remain undetectably low on day 26. Another participant (code: 6, 150 IU) had 2.9-times higher γ-tocopherol levels in plasma and 2-times higher in LDL after termination of the study than before, though he had undetectable levels of the γ-isomer from days 10–18 during the study

α-Tocopherol in LDL and resistance of LDL against oxidation

The LDL-α-tocopherol significantly increased in all participants in the experimental group during supplementation time (**Fig. 2** and Table 1). After a rapid initial increase, more or less plateau-like levels were reached in most cases. With daily supplementation of 1200 IU and in one case of 225 IU, no plateau was reached after 21 days. The increase of α-tocopherol in LDL was 2.7- to 3.1-times the initial level on day −2 with 1200 IU, 1.4- to 1.7-times with 800 IU, 1.8- to 2.3-times with 225 IU, and 1.4- to 1.7-times with 150 IU, respectively. No significant change was seen in the placebo group. The mean α-tocopherol value in the placebo group was 2.70 ± 0.42 nmol/mg LDL. α-Tocopherol rapidly decreased after termination of supplementation and reached values on day 26 that were only slightly above the initial values on day −2 (Fig. 2), except after supplementation with 225 IU where they were 23% higher than before. In one case (code: 6, 150 IU) α-tocopherol was 27% lower on day 26.

Fig. 3 shows the relationship between α-tocopherol in plasma and LDL. LDL-α-tocopherol increased with the plasma tocopherol, as expected (r^2 = 0.67). An increase of plasma α-tocopherol from 20 to 40 μM would give, on average, an increase of LDL- α-tocopherol from 2.6–4.3 nmol/mg LDL.

The oxidation resistance of LDL as measured by the duration of the lag-phase was significantly higher during the period of vitamin E supplementation (Fig. 2 and Table 1). The efficacy of vitamin E to prolong the lag-phase appears to vary from person to person as can be seen from the inserts in Fig. 2. For four subjects (1 × 150 IU, 2 × 800 IU, 1 × 1200 IU) the correlation between the lag phase and the α-tocopherol content of LDL was significant (r^2 = 0.74–0.89). In these subjects, each additional molecule of vitamin E per LDL particle caused an increase of the lag-phase of about 5 min. For four subjects (2 × 225 IU, 1 × 1200 IU, 1 × placebo) the correlation was less pronounced (r^2 = 0.48–0.60), and for the four others (three placebos and one × 150 IU) no correlation (r^2 = 0.06–0.28).

If all values for tocopherols and lag-phase obtained in this study (n = 84) are treated statistically, a correlation coefficient of r^2 = 0.51 is obtained (**Fig. 4**). This suggests that in addition to vitamin E other variables also influence

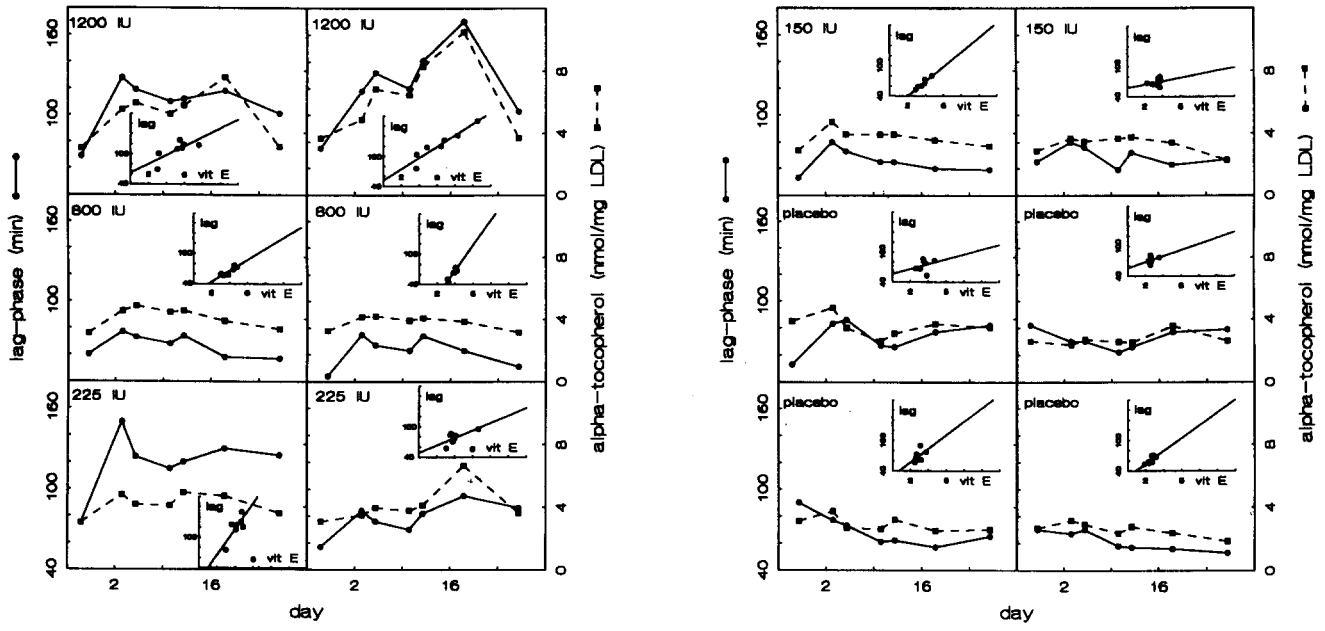

Fig. 2. Changes of α-tocopherol concentration and oxidation resistance (lag-phase) of LDL after daily administration of RRR-α-tocopherol. α-Tocopherol (■----■) and lag phase (●——●) were determined in freshly isolated LDL (as described in the Methods section) 2 days before, five times during, and 6 days after 21 days supplementation with RRR-α-tocopherol or placebos (concentrations are indicated in the left corner of each graph). α-Tocopherol was measured by HPLC and the lag-phase was determined from records of the 234 nm absorption during copper-mediated oxidation of LDL. Each graph gives the data of a single participant of the study. Inserts show the correlation between α-tocopherol (in nmol/mg LDL) and the associated lag-phase (in min) of the particular LDL fractions.

the oxidation resistance of LDL, which is consistent with the residual lag-phase of about 30 min extrapolated for an α-tocopherol-free LDL. It is intriguing to assume that this is determined, at least to some extent, by the other antioxidants present in LDL, e.g., carotenoids.

Carotenoids in plasma and LDL

Although this ex vivo study was primarily designed to measure the effect of RRR-α-tocopherol, we have included the determination of the carotenoids in plasma and LDL in order to obtain more information on their potential significance in protecting LDL against oxidation (**Fig. 5** and Table 1). The concentration of the carotenoids in plasma and LDL varied strongly not only between individuals but also in each individual case.

The mean plasma carotenoid concentrations calculated from the 84 determiantions performed in the study were: 0.57 ± 0.38 μM β-carotene; 0.22 ± 0.11 μM lycopene; 0.42 ± 0.27 μM cryptoxanthine; 0.23 ± 0.11 μM zeaxanthine/luteine. The mean values (n = 84) per mg LDL were: 0.107 ± 0.077 nmol β-carotene; 0.045 ± 0.027 nmol lycopene; 0.053 ± 0.035 nmol cryptoxanthine; 0.019 ± 0.010 nmol zeaxanthine/luteine. The reason for the high variability of carotenoids between LDL from different donors and among the LDL of one and the same donor at different times might be influenced to a high extent by dietary conditions. Neither in plasma nor in LDL was there seen an association between the carotenoid and vitmain E levels.

A summary of the data of vitamin E, carotenoids, cholesterol, and oxidation resistance of LDL of each single participant is also reported in Table 1 since, in our opinion, a comparison of these data gives further information (in addition to the figures) about the high variability of the measured parameters.

DISCUSSION

We and others have previously shown (10, 13) that heavy oxidation of the PUFA in LDL occurs only if the

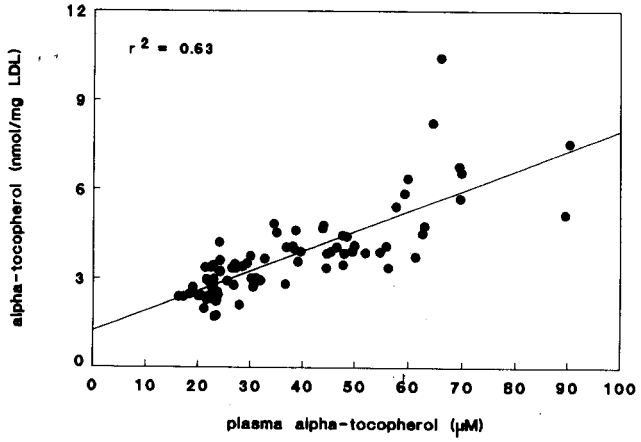

Fig. 3. Correlation between α-tocopherol in plasma and LDL. α-Tocopherol was measured in plasma and LDL of all participants by HPLC.

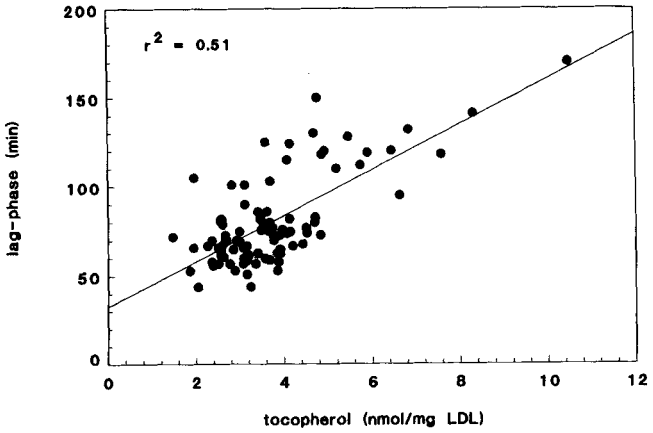

Fig. 4. Correlation between tocopherols of LDL and the corresponding oxidation resistance (lag-phase). Tocopherols (α- and γ-) were measured by HPLC and the resistance of LDL against copper-mediated oxidation was determined by recording the change of the absorbance at 234 nm.

endogenous vitamin E contained in LDL is exhausted. This has been found in cell-mediated oxidation (10) as well as in oxidation experiments with copper ions as pro-oxidants. This suggests, but does not necessarily prove, that α-tocopherol in LDL acts as an antioxidant. The fact that α-tocopherol is consumed before the onset of lipid peroxidation could simply result from a higher vulnerability of α-tocopherol to free radical attack as compared to the PUFA. Preliminary evidence that α-tocopherol truly acts as an antioxidant in LDL was obtained from in vitro studies in which LDL was isolated from vitamin E-supplemented plasma (13). Here, LDL with widely different α-tocopherol content could be prepared and a strict linear relationship existed between the oxidation resistance and the α-tocopherol content of in vitro-enriched LDL particles. One in vivo case has so far been reported in which a volunteer took 1.45 g (2160 IU) α-tocopherol daily 3 days before blood donation (10). The LDL preparation was remarkably more resistant towards oxidation as compared to the corresponding control LDL. Although these previous studies ascribe vitamin E an important protective role, it has been found that the vitamin E content of normal LDL (from plasma of unsupplemented subjects) does not allow one to predict the oxidation resistance of a particular LDL preparation (10, 13, 25). In fact, if we treat statistically the data of lag-phases measured before the study (on day − 2) together with all the data of the placebo group (n = 36) with the corresponding α-tocopherol values, the resulting correlation coefficient is only r^2 = υ.145. This weak correlation, however, should not be interpreted in a way that suggests that vitamin E is not an important protective factor in LDL. Together with the in vitro results, one must rather assume that the oxidation resistance of LDL depends on more than one variable. In LDL samples with average vitamin E contents of 2.7 ± 0.42 nmol/mg LDL, the contribution of vitamin E to the total oxidation resistance appears to be weak; this means that the other variables are of greater importance. Through the supplementation we could significantly increase the vitamin E content of LDL above the normal range in all subjects participating in the study (Fig. 2). The oxidation resistance of LDL increased in all participants; however, the response was different in each individual case (inserts in Fig. 2). In our opinion this suggests that the efficacy of vitamin E in protecting LDL against oxidation varies at least to some extent from person to person.

Nevertheless, with the large number of data that we have accumulated in this study, the median vitamin E effect can be separated statistically from all other variables as shown in Fig. 4. The correlation coefficient of r^2 = 0.51 means that, in a group of subjects with LDL vitamin E contents in the range of 2 to 10 nmol/mg LDL, about 50% of the oxidation resistance is mediated by vitamin E. Interesting in this respect is a cross-cultural epidemiologic study in the European population that revealed a significant inverse correlation between the mortality by ischemic heart disease and the levels of vitamin E in plasma (19). In this study the risk for ischemic heart disease was inversely correlated with vitamin E with r^2 = 0.55. In the investigated population the plasma α-tocopherol content varied from about 20 to 50 μM which corresponds, according to our measurements (Fig. 3), to about 2.6–4.6 nmol vitamin E/mg LDL.

Our results for plasma generally agree with those of Kitagawa and Mino (22) who found maximum levels of α-tocopherol in plasma that were 2.5- to 3-times the baseline values after administration of 900 IU of RRR-α-tocopherol to 14 students for 12 weeks.

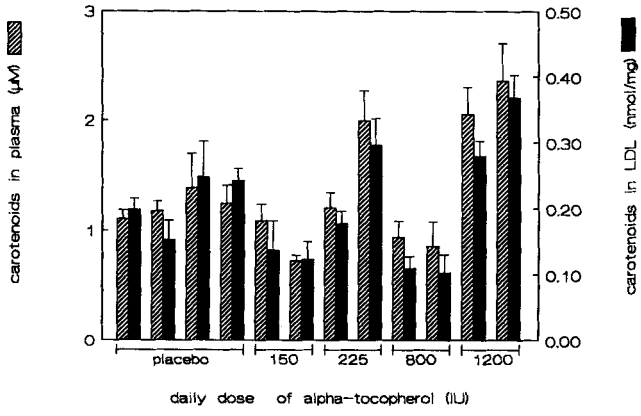

Fig. 5. Effect of dietary intake of RRR-α-tocopherol on the sum of carotenoids in human plasma and LDL. Carotenoids (β-carotene, lycopene, cryptoxanthine, and zeaxanthine-luteine) were determined by HPLC in freshly prepared plasma and LDL before (day − 2), five times during (on days 3, 5, 10, 12 and 18), and 6 days after oral supplementation with RRR-α-tocopherol. Values express the mean ± SD during supplementation time from each participant of the study.

The fact that LDL of vitamin E-supplemented subjects showed a higher resistance against oxidation in vitro suggests, but does of course not fully prove, that the same is applicable for the in vivo situation. Although the half life of LDL in plasma is about 2.5 days before it is cleared by the liver, it seems rather unlikely that LDL could become oxidized in plasma during this period to such an extent that it leads to foam cell formation and possesses chemotactic and cytotoxic properties. This is because plasma is rich in water-soluble antioxidants such as ascorbate, urate, and others. It has been documented by numerous in vitro studies (13, 23) that ascorbate has a protective effect on vitamin E, since it efficiently recycles vitamin E: vitamin E-radical + ascorbate → vitamin E + ascorbyl radical. A depletion of α-tocopherol from LDL, which is required for heavy lipid peroxidation in LDL, is probably not possible. On the other hand, it is conceivable that areas exist in the arterial wall where the water-soluble antioxidants are destroyed, e.g., by oxygen radicals released from inflammatory cells. LDL present in such an environment would fully depend on the protection by its own antioxidants. This is the situation where we believe that an increased α-tocopherol content of LDL could become a crucial parameter.

Our previous studies have also suggested that carotenoids are important components concerning the oxidation resistance of LDL (7, 9). This was in part confirmed in the present study by the fact that the lag-phase showed a higher correlation with total antioxidants than with α-tocopherol alone. If the lag-phase is correlated with the total antioxidant content of LDL, i.e., vitamin E plus all carotenoids, a correlation coefficient of $r^2 = 0.60$ results (**Fig. 6**) which has to be compared with $r^2 = 0.51$ if the lag-phase is correlated with α-tocopherol only (Fig. 4). This indicates that the carotenoid content of LDL is a component that is positively correlated with the oxidation resistance. There are certainly other components that are of equal if not greater importance. It has been reported for example that LDL with a higher content of oleic acid (24) is significantly more resistant to oxidation compared to LDL rich in linoleic acid. Other important components might be endogenous preformed lipid hydroperoxides that would reduce the oxidation resistance, or other antioxidants such as ubiquinol that would increase the oxidation resistance (21).

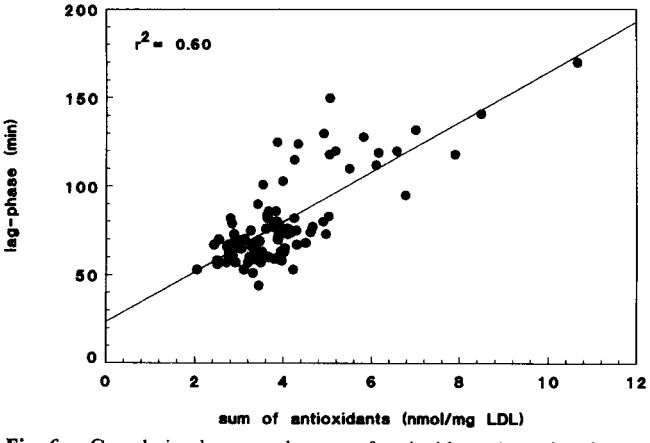

Fig. 6. Correlation between the sum of antioxidants (tocopherols and carotenoids) of LDL and the corresponding oxidation resistance of (lag-phase). Antioxidants and lag-phase were determined as described in the Methods section.

It was not possible in this study to determine the relative importance of the various carotenoids. For that it would be necessary to vary these components in LDL in a wider range either in vitro or by in vivo supplementation, for example, with β-carotene.

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